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ORIGINAL ARTICLE

Post-fire Quercus mycorrhizal associations are dominated by *Russulaceae*, *Thelephoraceae*, and *Laccaria* in the southern Appalachian Mountains

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Abstract

Following disturbances such as wildfires, oak seedlings must form a symbiotic association with mycorrhizal fungi to survive. Wildfires, however, reduce available mycorrhizal fungal propagules in the soil. Ectomycorrhizal (ECM) fungi on oak seedlings sampled in severely burned (7 sites), moderately burned (7 sites), and unburned areas (8 sites) in the Great Smoky Mountains National Park were evaluated 21 months after the 2016 Chimney Tops 2 Wildfire by Sanger sequencing of the nuclear ribosomal DNA internal transcribed spacer region (nrITS; fungal barcode). Sequences were aligned and grouped into Operational Taxonomic Units (OTUs) based on well-supported phylogenetic clades and 98–100% nrITS sequence homology with sequences in GenBank. One hundred seventy-nine root-associated fungi comprising 124 OTUs were recovered after removing duplicates (the same fungus on two or more roots of the same plant). The ECM genus *Russula* was the most diverse genus (25 OTUs), followed by the *Thelephora*/*Tomentella* clade (18 OTUs), *Lactifluus* (8 OTUs), *Lactarius* (4 OTUs), and *Laccaria* aff. *laccata* (2 OTUs). *Russula* OTUs were identified more frequently on oak roots from burned areas and in burned soils, suggesting that some *Russula* taxa may have a selective advantage in burned areas. High alpha diversity occurred within each of the burn categories, but little overlap of taxa occurred between burn categories (high beta diversity). Approximately half of the recovered OTUs (100/179 total root-associated fungi = 55.9%) were found on a single plant. Oak seedlings growing in moderately and severely burned areas 21 months after a fire were capable of forming root associations with available fungi. In contrast to the expectation that root-associated fungal diversity would be reduced after a wildfire, diversity 1 year after the Chimney Tops 2 Fire was high with ectomycorrhizal *Laccaria*, *Russulaceae*, and *Thelephoraceae* dominating. This study suggests that the availability of ECM fungi post-fire is not a barrier to oak re-establishment.

Keywords Community ecology · Fungal diversity · Ectomycorrhiza · Species richness · Wildfire recovery

Background

Wildfires are an essential component of ecosystems worldwide (Marlon 2020). Many ecosystems are dependent on wildfires, but the frequency and intensity of wildfires have varied over time (Calder et al. 2015; Hély et al. 2020; Hoecker et al. 2020). In the Southern Appalachian Mountains (Tennessee and North Carolina), and in forests of the eastern USA, fire prevention strategies have successfully suppressed forest fires for approximately 100 years (Nowacki and Abrams 2008; Aldrich et al. 2010; Flatley et al. 2011, 2013; Lafon et al. 2017). In the Great Smoky Mountains National Park (GSMNP), fires have been largely suppressed since the Park was established in 1934 (Flatley et al. 2011,

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2013). More recently, prescribed fires (Rx fires) have been selectively used to reduce fuel loads and protect nearby urban areas outside the GSMNP boundaries. Recently prescribed fires were included in this study for comparison.

Wildfires are reported to reduce both the number of ectomycorrhizal (ECM) fungi and their diversity (Tuininga and Dighton 2004; Buscardo et al. 2010, 2012; Glassman et al. 2015; Pérez-Izquierdo et al. 2020; Fox et al. 2022), but this is dependent on the severity and extent of fire disturbance. The importance of ECM fungi to forests in providing plants with essential nitrogen and phosphorus cannot be underestimated (Marschner and Dell 1994, Högberg et al. 1999). While many ECM fungi are removed by wildfires, some may survive on deep surviving roots, and a few are resistant to fire, fruiting prolifically weeks or months after a burn (Hughes et al. 2020a). New ECM inoculum may also be acquired from adjacent unburned areas.

In late November and early December 2016, a wildfire burned natural forested areas in the Great Smoky Mountains National Park (GSMNP, USA) and destroyed parts of the town of Gatlinburg, Tennessee. The fire Chimney Tops 2 Fire (CT2) was exacerbated by unusually dry conditions following an extreme fall drought, was patchy, with unburned areas often adjacent to burned areas, and varied in fire intensity and damage resulting from variations in plant and soil moisture, topography, and local fuels. In 2017, no oak seedlings were observed in either moderately burned or severely burned areas, but at the end of 2017, mature *Quercus* trees in moderately burned areas dropped acorns that germinated in the spring of 2018. In severely burned areas, some oaks with dead trunks produced shoots and new fine roots from deep roots that survived the fire (i.e., coppicing), but seedlings germinating from acorns were very rarely observed, and the source of the acorns was unclear.

This study was undertaken to evaluate post-fire ECM and root-associated fungi on germinating 2018 oak seedling roots and on roots of coppicing oaks. Oaks are obligately ectomycorrhizal (Smith and Read 1996). When acorns germinate, they initially produce a non-mycorrhizal taproot. Subsequently, they produce lateral roots near the top of the taproot that become colonized with ECM fungi over time (Southworth 2012). Seedlings without mycorrhizal fungi may survive 1 or 2 years, but those that do not acquire appropriate ECM fungi do not thrive; saplings that survive are consistently mycorrhizal (Southworth 2012). We hypothesized that we would find reduced ECM and root-associated fungal species richness on oak roots in burned areas compared to unburned areas, consistent with reports that wildfire reduces the number and variety of ECM fungi in the soil (see references above). We included oak seedlings from both the areas burned by the 2016 wildfire and two areas that resulted from controlled burns in the eastern part of the GSMNP (Rx fires). We also hypothesize that ectomycorrhizal and root-associated fungi from prescribed burns would differ from those recovered in the 2016 CT2 wildfire burn areas.

To evaluate these hypotheses, we examined species richness and diversity of ECM fungi on 2018 (first year) oak seedlings from burned and unburned sites and from new secondary roots of coppicing oaks regenerating in severely burned sites.

Methods

Site selection

Site details are given in Supplementary materials Fig. S1 and Table S1. The uneven nature of the CT2 fire created a patchwork of burn categories, from unburned to high severity burn areas. For purposes of this study, low/moderately burned sites tended to be fast-moving ground fires on flat ground or the lower-middle portions of a slope. Generally, such fires removed dry fuels and leaves, some of the organic soil layer and scorched the base of trunks of mature trees (basal tree scorch up to 1.5 m high). Severely burned sites were fast-moving very hot fires, usually on slopes and ridge tops and involving *Pinus pungens* (Table Mountain pine) and *Pinus rigida* (Pitch pine), *Rhododendron maximum*, *Kalmia latifolia*, and *Quercus montana* (Chestnut oak). These were stand-replacing fires with significant tree death ($> 70\%$) and removal of the soil organic layer leaving a hard clay layer exposed. The Baskins Creek Trail in the Great Smoky Mountains National Park (GSMNP) was selected for the close proximity of three different burn categories (unburned, moderately burned, and severely burned). The remaining survey areas were established using a GIS fire severity raster map with a 30 m resolution generated by the US Forest Service Remote Sensing Application Center (Beals et al. 2022). Candidate field sites within each burn severity classification (unburned, moderately burned, and severely burned) were randomly selected. Three 90 m^2 blocks within each burn severity classification located within 300 m from roads and park trails for ease of accessibility were selected. Sites were verified for differences in burn severity by on-the-ground observations (Supplementary materials, Table S1).

Two prescribed fire areas were included in the study, Lynn Hollow and Arbutus Ridge (Supplementary Materials Fig. S1 and Table S1). Both contain sites that could be considered low/moderate and severely burned. Lynn Hollow was burned in 1999 and 2014. Areas of severe damage were due to accumulation of dry fuels following a severe southern pine beetle outbreak that resulted in loss of mature yellow pine including some old-growth shortleaf pine. The Arbutus Ridge area was burned in 2003, 2007, and 2015/2016. This was primarily a low/moderate burn but with patches of severely burned forest. Collections in both sites were from both low/medium and severe burns. Unburned controls were from nearby sites that were outside the fire boundaries (Supplementary materials, Table S1).

Collections

Three to four first-year oak seedlings (Fig. 1a) were removed from each of 22 sites with additional seedlings from two sites with a high density of seedlings. Oak seedlings varied by site but included primarily white oak (*Quercus alba* L.), red oak (*Q. rubra* L.), and chestnut oak (*Q. montana* Willd.). A few collections of black oak (*Q. velutina* Lam., a species in the red oak group) were made. Additionally, roots (Fig. 1b) at the base of coppicing chestnut oaks (Fig. 1c) were removed by digging fine roots by hand that appeared to be attached to the base of burned trunks or attached to lateral roots in the top 2–3 inches. In severely burned *Pinus pungens*-Chestnut oak areas, the soil lacked an organic layer and was difficult to penetrate because of the mass of surface roots exposed by the fire and the packed nature of the soil. Because of difficulties associated with sampling dense root-bound soils, and GSMNP restrictions on collections, we did not sample deeper roots and acknowledge that, in the case of coppicing *Q. montana*, we cannot be certain that all of the sampled roots are oak (9/179 collections). Seedlings and roots were transported on ice to laboratories at the University of Tennessee where each seedling or set of coppicing roots was assigned a “fire survey number” representing a

Fig. 1 Experimental materials: **a** Oak seedling which germinated in the spring of 2018 and was collected during the summer of 2018, approximately 20 months after the Chimney Tops 2 Wildfire. **b** Oak seedling roots. **c** Coppicing chestnut oaks in severely burned oak-pine habitat

single plant. Plants were photographed. Roots were washed free of soil and fine roots were examined microscopically for the presence of fungi. The numbering of putative ectomycorrhizae from each plant consisted of the plant fire survey number followed by a letter to indicate the root tip that was sampled (Supplementary materials Table S2). Root tips that were expanded and appeared to be covered with a fungal mantle were photographed using a Leica EZ4W dissecting microscope/camera (Leica Microsystems, Buffalo Grove, Illinois, USA), then removed for subsequent DNA extractions.

Molecular procedures

DNA was extracted from each putative ectomycorrhizal root tip using a modified rapid DNA extraction procedure (Hughes et al. 2020b). PCR amplifications of the fungal barcode (Schoch et al. 2012), the nuclear ribosomal internal transcribed spacer (nrITS = nuclear ribosomal ITS1-5.8S-ITS2) region, used combinations of PCR primers including ITS1F, ITS2, ITS3, and ITS4 (White et al. 1990; Gardes and Bruns 1993). We also attempted PCR amplification of the 5' end of the nuclear ribosomal large subunit (nrLSU or 28S) using primers LR0R and LR5 (Hopple and Vilgalys 1994) but successful nrLSU amplifications were rare and were not used to assign OTU designations. Sequencing was carried out at the University of Tennessee Genomics Core. Tentative fungal identifications were based on 98–100% GenBank blast matches of nrITS sequences to identified taxa represented by herbarium specimens. In some cases, GenBank blast matches ≥ 99% were to unidentified environmental sequences. In this case, the oak mycorrhizal fungus sequence was assigned to an OTU representing both the oak mycorrhiza and the GenBank matches. If no GenBank match was found (defined as less than 98%), an OTU specific for the unknown ITS sequence was also assigned. For speciose taxonomic groups (*Russula, Lactifluus, Thelephora-Tomentella*), all sequences were aligned in AliView (Larsson 2014) and evaluated for clade independence by PhyML in Geneious 11 (Guindon et al. 2010). Clades that were well-supported by bootstrap values, with an internal similarity greater than 97% and with clade-specific apomorphies were accepted as an OTU.

Data management

When two or more root tips from the same plant had the same nrITS sequence (i.e., the same OTU), duplicates were removed from subsequent analyses of taxonomic diversity on the assumption that these were not independent events but a consequence of the same fungus inhabiting more than one root. The data set for analysis therefore represents OTUs/ per plant.

We were able to match a DNA sequence to a taxon name or an OTU in most cases (Supplementary Materials Table S2), even when the sequence contained ambiguities or only a partial nrITS sequence was available. OTUs and named species were coded with a four-digit code (e.g., *Russula*: Ru01, Ru02). Genera were assigned to families using Index Fungorum (www.indexfungorum.org) and GenBank. In five cases, a nrITS sequence resulted in a different species designation (OTU) than that based on LSU sequences. These may represent instances where both species were present on a root sample (Morris et al. 2008a), but where PCR bias occurred for one or the other taxon during PCR amplification. In these cases, both OTUs were included in the data set.

Within *Russula*, only 8 of 25 OTUs could be assigned a name based on a 98% or better match to a named sequence. In three cases GenBank accessions were deposited under more than one name by knowledgeable investigators. In other cases, OTUs that were clearly separated by nrITS sequences were listed under the same name (*Russula pectinatoides* Peck, *R. cyanoxantha* (Schaeff.) Fr. and *R. puellaris* Fr.). Since physical types for many of these taxa do not exist and neotypes or epitypes have not been established, these ambiguities cannot be resolved and we therefore continue to use OTU designations.

We compared the alpha and beta diversity of EMC species on oak roots as a function of fire severity and burn type (prescribed versus wildfire) with the following diversity estimates.

Richness

Species richness is defined herein as the number of species in a sample (the total number of fungal taxa/OTUs identified from an oak root tip. In contrast, species diversity is some measure of the number of taxa and the number of individuals (Spellerberg 1991).

Distance-based redundancy analysis (dbRDA) (Legendre and Anderson 1999) is a multifactorial analysis of variance technique that determines the principal components of multispecies response variables. Permutation tests for dbRDA were performed in the R Vegan package (Oksanen et al. 2024) implemented in R 4.3.2 (R_Core_Team. 2021) for tree species and fire intensity. Permutations = 999.

Alpha diversity estimates

Alpha diversity estimates (Shannon Index, Shannon Exponent, Simpson, and Chao1) between fire regimes were generated using EstimateS (Colwell 2013). The Shannon Index (H') was also calculated manually as a check. Species accumulation curves were generated using iNEXT online [https://chao.shinyapps.io/iNEXTOnline/ (Chao, A. 1984)] with q = 0, which disregards species abundances (Chao et al. 2020).

Jaccard similarity coefficient

The Jaccard coefficient (Jaccard 1912) is a measure of beta diversity and ranges between 0 and 1 with 0 indicating no overlap and 1 indicating complete overlap between two data sets. The Jaccard similarity coefficient was calculated for taxa in each of the three burn categories from the formula $J(X, Y) = |X \cap Y| / |X \cup Y|$ where X and Y are the number of taxa (OTUs) in each of the burn categories. The significance of the coefficients was determined from tables in Real (1999).

Betadispr (Vegan) [multivariate homogeneity of groups dispersions]

Betadisper is a multivariate test for homogeneity of variances. Betadispr tests whether two or more groups are homogeneously dispersed with respect to their species composition, an estimate of beta diversity. Betasdispr was used to test whether variances of fungal root taxa in different regimes were homogeneous. The statistical significance was estimated by ANOVA. A comparison of the means of fire intensity was performed using "TukeyHSD".

Results

Quality

Oak mycorrhizae and other associated root fungi were concentrated on fine roots which formed at the top of the tap root. Fine roots were rare lower on the tap root. Oak mycorrhiza lacked a well-developed mantle (Fig. 1b). It was rare to obtain a strong PCR product from single oak mycorrhizal root tips using nrITS primers ITS1F and ITS4. More commonly, nrITS PCR products were obtained using primers ITS1F in combination with ITS2, and ITS3 in combination with ITS4 or ITS4b. Sequence trace files often featured multiple overlapping peaks reflecting the likely presence of more than one root-associated fungus or a contaminating oak sequence. Contaminating oak sequences (or in some cases, clean oak sequences) were more frequent with the ITS1F-ITS4 and ITS1F-ITS2 PCR amplifications than the ITS3-ITS4 amplifications. We hypothesize that contaminating oak sequences were likely due to the quantity of fungal tissue vs. oak root tissue in a given sample. LSU sequences were rarely successful.

PCR amplification success

We collected roots from a total of 109 oak seedlings or new fine roots from coppicing oaks across 22 sites in the GSMNP comprising 361 root tip samples. Of these, 238 (65.9%) produced a sufficiently clean sequence for tentative fungal identification. Of root tips which did not produce any PCR product, 39.8% were from severely burned areas, 28.7% from moderately burned areas, and 29.7% were from unburned areas. The higher failure of PCR amplifications from severely burned areas may reflect the effects of altered soil parameters in the severely burned areas on the formation of mycorrhiza on newly grown fine oak roots. For comparison, García-Guzmán et al. (2017) reported a 34% PCR success rate in roots samples from unburned Oak forests in Mexico and Walker et al. (2005) reported a 49% PCR success rate from unburned forest in Coweta, North Carolina.

Number of OTUs (rarity and richness)

There were 124 OTUs / 236 identifiable root tip PCR amplifications. *Russula* was the most diverse genus (25 OTUs/124 or 20.1%) followed by the *Thelephora-Tomentella* clade (18 OTUs/124 = 14.5%), *Lactifluus* (8 OTUs/124 = 6.5%), and *Lactarius* (4 OTUs/124 or 3.2%) (Supplementary Materials Table S2). *Russula*, *Lactarius*, and *Lactifluus* are closely related genera in the family *Russulaceae*. The diversity of OTUs recovered does not give the whole picture, however. Some OTUs were common on oak roots and some were rare (Supplementary Materials Table S2). In contrast to the high taxonomic diversity of *Russula* found on oak roots, only two species of ECM *Laccaria* (*Hydnangiaceae*) were identified on oak roots but were recovered frequently: *Laccaria* aff. *laccata* was recovered on the roots of 13/106 (12.3%) oak seedlings located in severe or low/moderate fire zones but never from oaks in unburned areas. *Laccaria trichodermophora* was recovered from 4 seedlings, 3 from severe fire zones, and one from an unburned area. Sporocarps of *L.* aff. *laccata* were common in low-moderate fire zones; *L. trichodermophora* sporocarps were common and persistent only in severe fire zones.

Ninety-six OTUs (Supplementary Materials Table S2) were represented by a single root tip (Rarity). Several taxa recovered from oak roots have not been reported as ECM (e.g., *Delicatula integrella, Gerronema strombodes, Leotia lubrica*). In these cases, the PCR process likely amplified root-associated taxa with a variety of root affiliations. *Delicatula integrella* has been isolated from *Fraxinus excelsior* roots in Germany (Lysenko et al. 2024) and found growing on oak tree roots in the GSMNP (Grootmyers, D. Pers. Comm.). *Leotia lubrica* has been confirmed as arbutoid mycorrhizal with members of *Ericacea*e which were common in burn areas before the wildfire (Kühdorf et al. 2015; Tedersoo and Smith 2017).

Species accumulation curves

Species accumulation curves are given in Fig. 2. Species accumulation curves did not level off suggesting that the total available taxa were not sampled. This may be due to limitations on the number of seedlings sampled at each location. There was little difference between control (unburned) areas and areas with moderate burn intensities.

Alpha diversity

Alpha diversity metrics (Shannon, Shannon exponential, Simpson Inverse, and Chao1) are given in Table 1. The highest actual richness in terms of total species in each category was in the low/moderate-burn category. Both Shannon H', which assumes all species are represented in a sample and that they are randomly sampled, and the Simpson Inverse Distribution, which gives more weight to dominant species, indicate the highest diversity is in the low/moderate burn category. The Shannon exponential mean gives the Effective

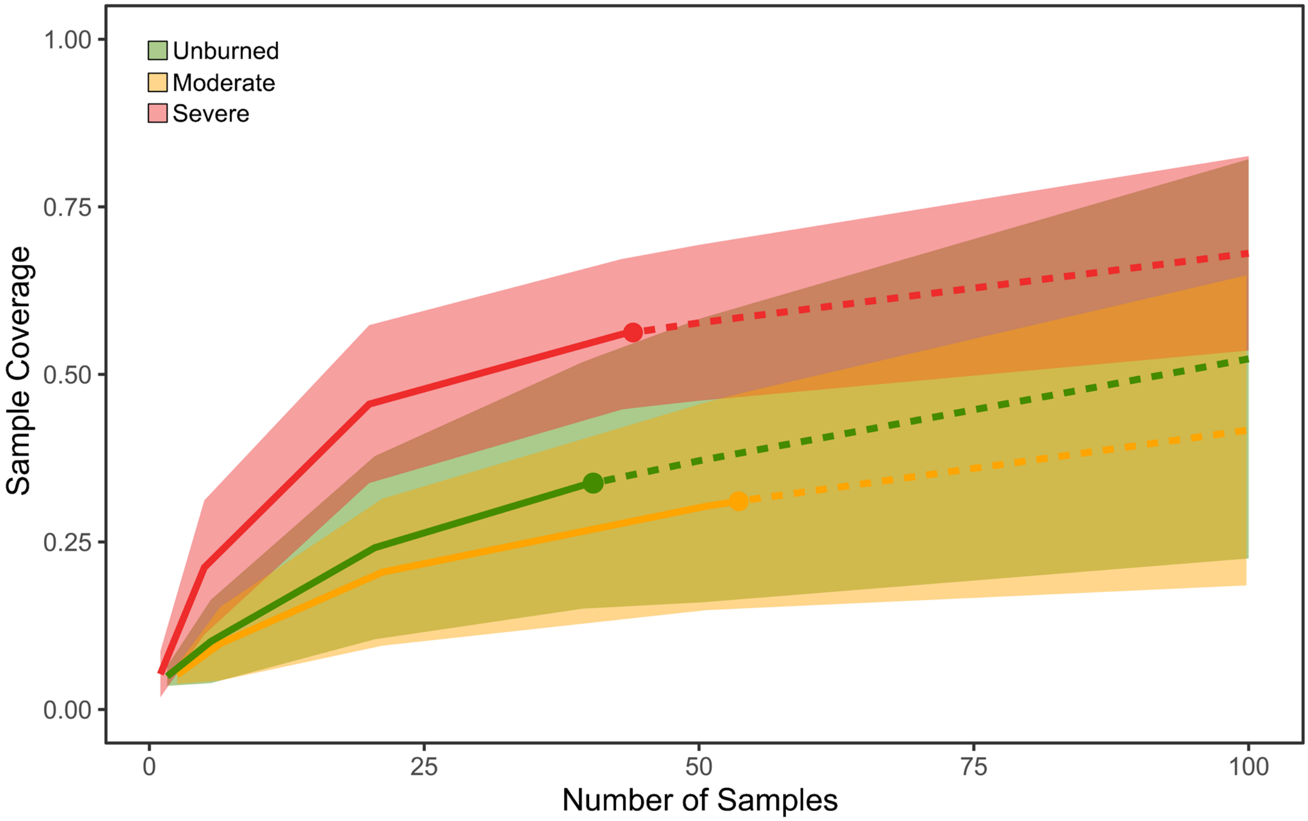

Fig. 2 Rarified (solid lines) and extrapolated (dashed lines) species accumulation curves for differing burn categories with 95% confidence limits

Table 1 Richness and alpha diversity metrics

Metric	Unburned	Low/moderate burn	Severe burn
Richness	41[1]	53[1]	44[1]
Shannon H' (Hill number q = 0)	3.6 (0.72)[2]	3.83 (0.81)[2]	3.49 (0.74)[2]
Shannon exponential mean (Hill number q = 1)	36.61	46.16	32.70
Simpson Inverse diversity (Hill number q = 2)	32	37.09	22.96
Chao1	239.91 ± 134.5	312.3 ± 151.96	178.31 ± 82.65

[1] Taxa in more than one burn category are included in each category in which they appear

[2] Dividing Shannon diversity indices for the three fire severity groups by the Shannon total diversity gives a metric between 0 and 1 (parentheses)

Species Number (ESN) and is highest in the low/moderate burn category. Chao1 which emphasizes rarity gives more weight to rare species is also highest in the low/moderate burned category.

At the species/OTU level, there was little overlap of species in the different burn categories. A Venn diagram (Fig. 3) of taxa at the species/OTU level showed that most recovered taxa were restricted to specific burn categories. Only two taxa were shared across all sites, Ru17 (*Russula cyanoxantha*) and Cn01 (*Cenococcum geophilum*) which is ubiquitous (Stendell et al. 1999; de Román and de Miguel 2005; Buscardo et al. 2010; Kipfer et al. 2011). Unburned and severely burned sites shared a single unique taxon (*Laccaria trichodermophora*) and two ubiquitous taxa, *Russula* 17 (Ru17) and *Cenococcum geophilum* (Cn01). Severely burned and moderately burned sites shared six taxa other than the two ubiquitous taxa (Fig. 3).

Distance-based redundancy analysis (dbRDA)

The fungal communities of oak species (white, red, black, and chestnut oak) did not vary significantly between

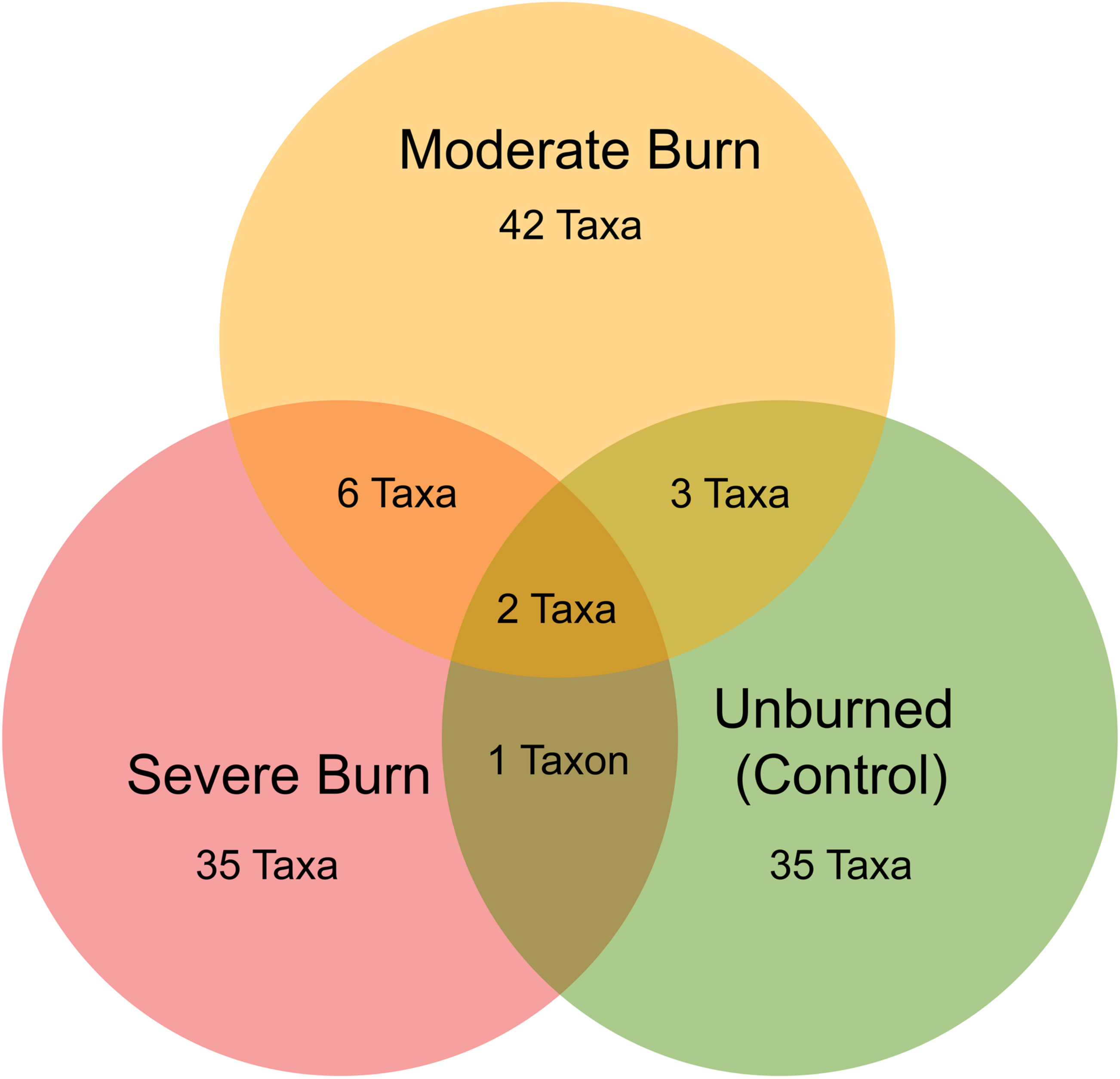

Fig. 3 VENN diagram of species/OTUs in different burn categories and overlap in composition among burn categories

tree taxa ($F=1.022$, $P=0.378$, DF = 3); however, the fungal communities differed among all fire severity categories ($F=1.717$, $P=0.019$, DF = 2), consistent with Fig. 3. Thus, the fungal taxa of unburned, moderately burned, and severely burned sites can be considered significantly different. Further, fungal taxa of CT2 wildfires and prescribed wildfires were also significantly different ($F=6.006$, $P=0.019$, DF = 1, and Fig. 4).

Betadispr

The variance of root-associated fungi associated with oak seedlings in different fire categories differed significantly ($F=7.493$, $P=0.002$, DF = 2; Table 2). Within the fire intensity data space, severely burned sites were more heterogeneous (Fig. 5) while unburned and low/moderately burned sites tended to be more homogeneous. Prescribed fires and wildfires were also significantly different (dbRDA analysis:

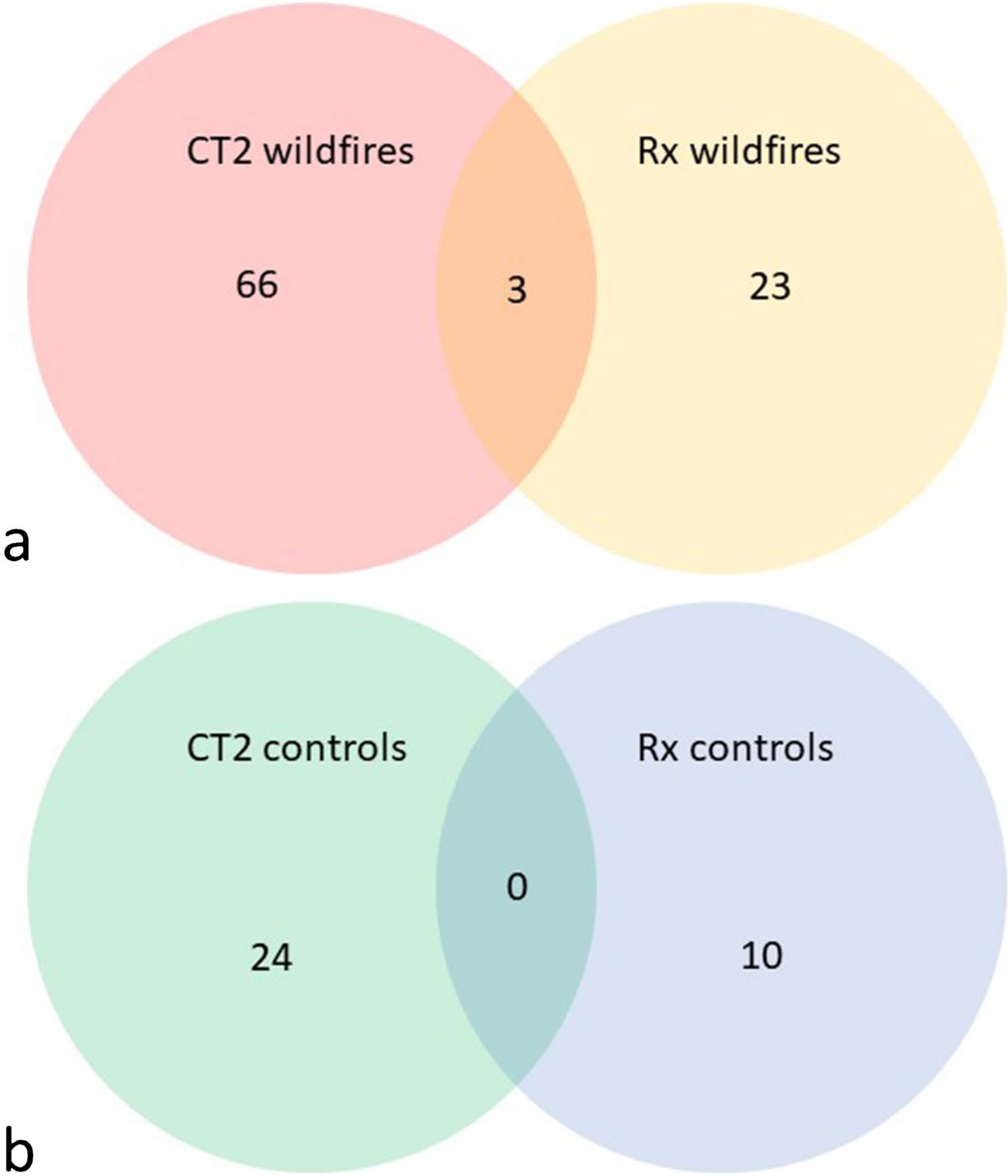

Fig. 4 VENN diagram of species/OTUs in CT2 wildfire areas vs prescribed fire areas. **a** Taxa from areas that were burned. **b** Taxa from unburned areas. CT2, Chimney Tops 2 wildfire sites; Rx, prescribed burn sites

Table 2 Results of dispersion analysis using Tukey multiple comparison of means

Group	difference	Lower 95% confidence limit	Upper 95% confidence limit	Probability (adjusted)
Low/medium vs. severe	0.01684507	−0.002371092	0.03606124	0.0956194
Unburned vs. severe	0.02880095	0.010650501	0.04695140	0.0011810**
Unburned vs. low/moderate	0.01195588	−0.005326110	0.02923786	0.2231707

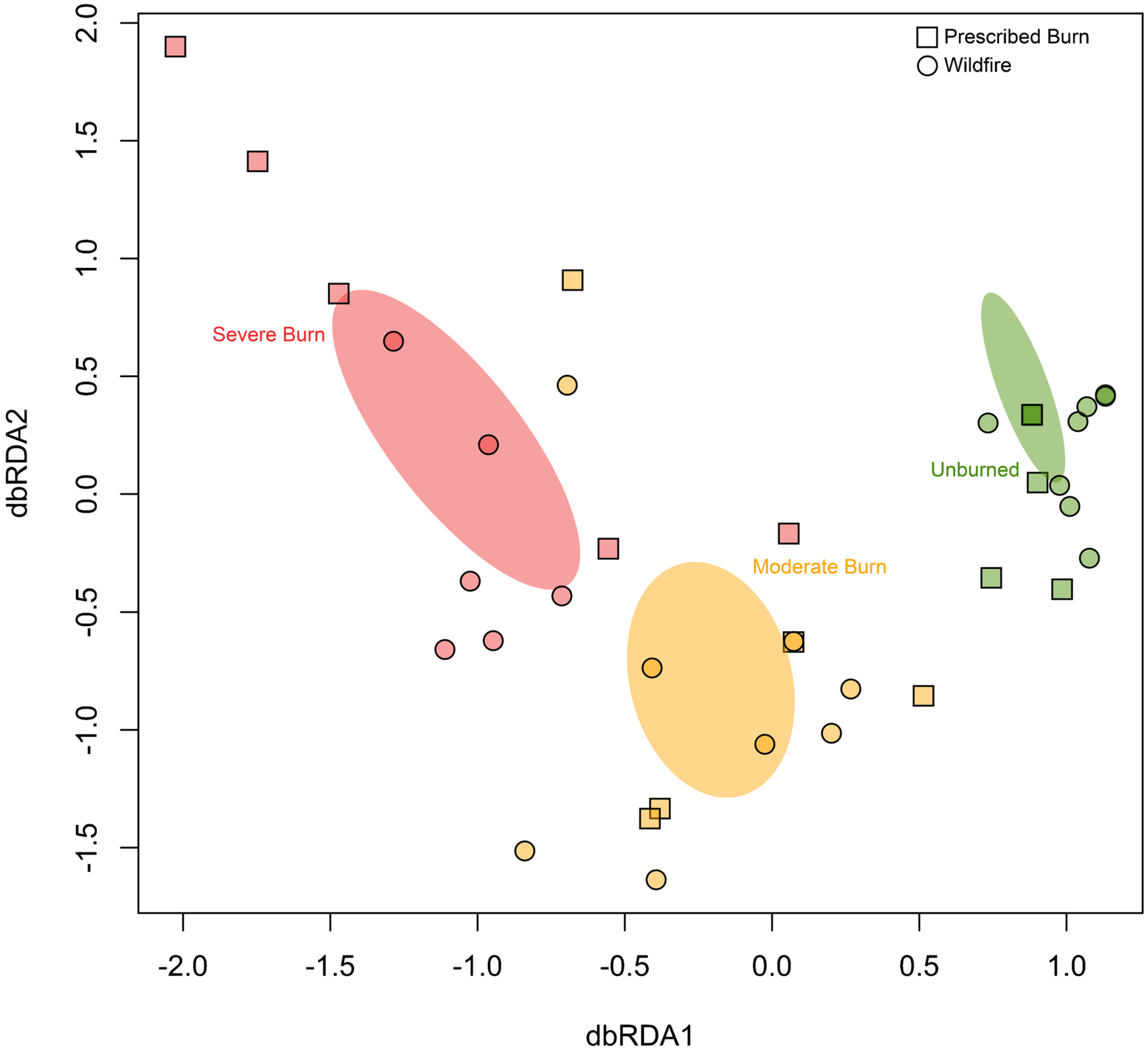

Fig. 5 dbRDA multivariate analysis for burn categories separated by tree species. Squares = prescribed burns, circles represent wildfires. Red = severe burns, yellow = low/moderate burns, and green = unburned adjacent control areas. Colored areas represent two standard deviations from the centroid

$F = 1.3964$, $P = 0.003$). Communities under prescribed burns were more homogeneous than wildfires based on the Betadispr analysis ($F = 6.006$, $P = 0.0189$).

A comparison of taxa in prescribed burns and CT2 wildfires showed only 3 shared taxa, *Xerocomus* aff. *chrysenteron*, *Lactifluus volemus* (Lf07), and *Lactifluus piperatus* (Lf05) out of 92 taxa isolated from low/moderate and/or severely burned sites. *Lactifluus volemus* and *Lactifluus piperatus* are common and wide-spread. If non-burn areas are compared between sites adjacent to the prescribed burn areas and the wildfire areas, there were no overlapping taxa (0/34 sites; Fig. 4).

Effects of burn severity on distribution of taxa

The distribution of mycorrhizal or root-associated families by burn severity is given in Fig. 6 and Supplemental Data Table S2. The most frequently recovered families were *Russulaceae* (*Russula*, *Lactarius*, and *Lactifluus*). While approximately equal numbers of *Russulaceae* were found in the three burn categories, there was very little overlap in species between these three groups. *Russula* OTUs Ru08 and Ru13 were also associated with severely burned areas. *Hydnangiaceae* (*Laccaria trichodermophora* and *L.*aff. *laccata*) were both associated with moderate and severe burns with *L. trichodermophora* fruiting prolifically on severely burned soil in association with *Pinus pungens* for 2 years after the Chimney Tops 2 wildfire (Hughes et al. 2020a). *Laccaria* is known to form mycorrhizal associations on oak (Southworth 2012). Mueller and Strack (1992) note that *L. trichodermophora,* normally a pine ECM in the GSMNP, underwent a host shift into neotropical oaks so finding *L. trichodermophora* on oak roots in this study is feasible. The *Thelephoraceae* primarily were associated with unburned and low/moderate burns. In severely burned areas, the *Porotheleaceae* are represented by a single species, *Delicatula integrella* which is not known to be mycorrhizal but has been found in Pecan root samples (Ren et al. 2023). This species has not been reported as biotrophic, and sporocarps have not been collected from the GSMNP but have been collected from east Tennessee. There was no apparent preference for burned sites for species within the *Thelephoraceae* (*Thelephora-Tomentella*), or *Russulaceae* (*Russula*, *Lactarius, Lactifluus*) other than Ru08 and Ru13.

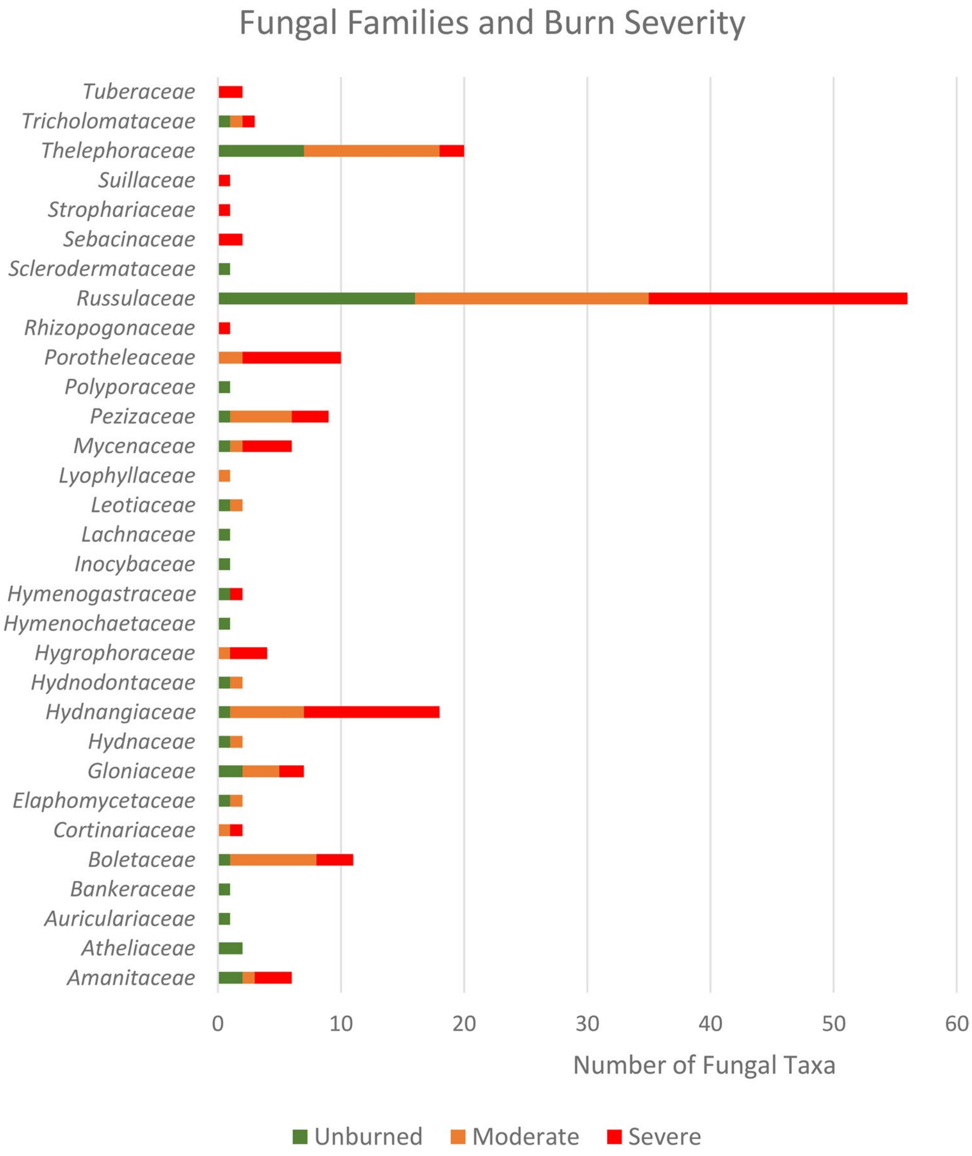

Fig. 6 Distribution of fungal families by fire severity

Several possibly non-mycorrhizal fungi were amplified from root tips including *Auriculariaceae* (1), *Hygrophoraceae* (4), *Hymenochaetaceae* (1), *Mycenaceae* (6), and *Polyporaceae* (1). In one of these, there were no close Blast matches and the genus was unknown (*Polyporaceae*). A second of these had a blast match only to unknown soil fungi (*Auriculariaceae*). The *Hymenochaetaceae* isolate had a blast match to *Coltriciella* sp. *Coltriciella* may include mycorrhizal taxa (Valenzuela et al. 2020). *Hygrocybe* is considered biotrophic, but the nature of the plant-fungal relationship is not well-defined (Seitzman et al. 2011; Halbwachs et al. 2013). While Mycena taxa are generally considered to be saprobic, there are a number of reports that *Mycena* species may associate with roots in a transitional state between saprotrophy and biotrophy (Thoen et al. 2020; Ogura-Tsujita et al. 2021; Pellitier and Zak 2021; Harder et al. 2024). Because the specific roles of individual OTUs identified in this study are often unknown but are root-associated, we have retained these in the data analyses.

Three unidentified truffle OTUs, *Tuber* Tu01 and Tu02 (*Tuberaceae*), two *Elaphomyces* OTUs, and *Rhizopogon pseudoroseolus* (*Rhizopogonaceae*) Rh01 (Supplemental Data Table S2) were recovered from root tips in severely burned areas. *Rhizopogon* is known to be associated with pine and was recovered from roots sampled directly under coppicing oaks at the base of the tree but we cannot exclude the possibility that the root was pine, not oak. *Elaphomyces* was recovered only from oak roots in unburned areas. In both cases, the underground propagules may have been protected against heat.

Effect of oak species on the distribution of ECM fungal OTUs

ECM fungal OTUs were evaluated independently for the three commonly collected oak species (*Quercus rubra*, *Q. alba*, and *Q. montana*). *Russulaceae* (*Russula, Lactarius, Lactifluus*) was present on roots of all three oak species at approximately equal frequencies (Fig. 7). Rare fungal OTUs, as might be expected, were often limited to one or two oak seedlings and thus appear to be limited to one oak species or another, but that is likely an artifact of sampling. The single exception could be *Hydnangiaceae* (*Laccaria* spp.) which was restricted to red and chestnut oaks and not recovered from white oak. A permutation test for plant species and fire intensity (dbRDA in R) did not find a significant difference in taxa due to tree species (see above: $F = 1.022$, $P = 0.378$, DF = 3).

Differences between coppicing roots and seedling roots

Coppicing oaks were predominantly located in severe fire zones so it was not surprising to find that species of mycorrhizal fungi or root-associated fungi from live roots of coppicing plants were the same as those included in Fig. 3 from high burn areas. *Russula* sp. 13 (Ru13) was more common on seedling roots from severely burned areas than from surviving roots from coppicing completely burned trees. *Delicatula integrella* and *Laccaria trichodermophora* were more common on roots from coppicing plants than from seedling roots. We cannot differentiate between effects due to fire severity vs. the physical process of new roots forming on coppicing stems instead of seedling roots. Our untested assumption is that new roots will associate with fungi in their environment and that fungal associations with new roots are independent of the source of the roots.

Discussion

Richness

The southern Appalachian Mountains, including the Great Smoky Mountains National Park (GSMNP), is a center of biodiversity for North America (Stein et al. 2000) with over 5000 documented species of fungi recorded to date from the GSMNP alone (MyCoPortal 2020). In this study, we evaluated the diversity of ectomycorrhizal fungi on oak seedlings 1 year after a wildfire in the GSMNP. Based on 238 successfully sequenced root tips, total richness was 124 OTUs with 96 (77.4%) recovered only once. In order to determine if the observed richness in this study was normal for the southern Appalachian mountains, we compared these results with an earlier study by Walker et al. (2005), who evaluated the diversity of oak seedling ECM fungi at the unburned Coweeta Hydrologic LTER site in the southwestern corner of North Carolina (also part of the southern Appalachian Mountains). Walker et al. (2005) out-planted 4-month-old seedlings of two species of Oak [*Quercus rubra* and *Q. montana* (formerly *Q. prinus*)] and analyzed ECM fungi on root tips 1 year later. As in this study, identifications were made by sequencing the nrITS region. Total richness was 75 OTUs (25.77%) with 42 OTUs (56%) having a frequency of only one (Table 3). One possible explanation for the markedly increased richness (diversity) identified in this study compared to Walker et al. (2005) may be due to a difference in the size of the sampling area. The Walker et al. (2005) study was limited to two ecosystems within Coweeta. The geographical scope of sampling in this study, while limited to the GSMNP, encompasses a broader diversity of ecosystems and a larger geographical footprint. To illustrate, there were no shared fungal ECM and root-associated taxa from unburned plots in the north-central region of the GSMNP and the eastern part of the GSMNP (Fig. 4). Suz et al. (2014) evaluated oak mycorrhiza across 22 long-term oak plots in 9 European countries (ICP Forests) which varied across

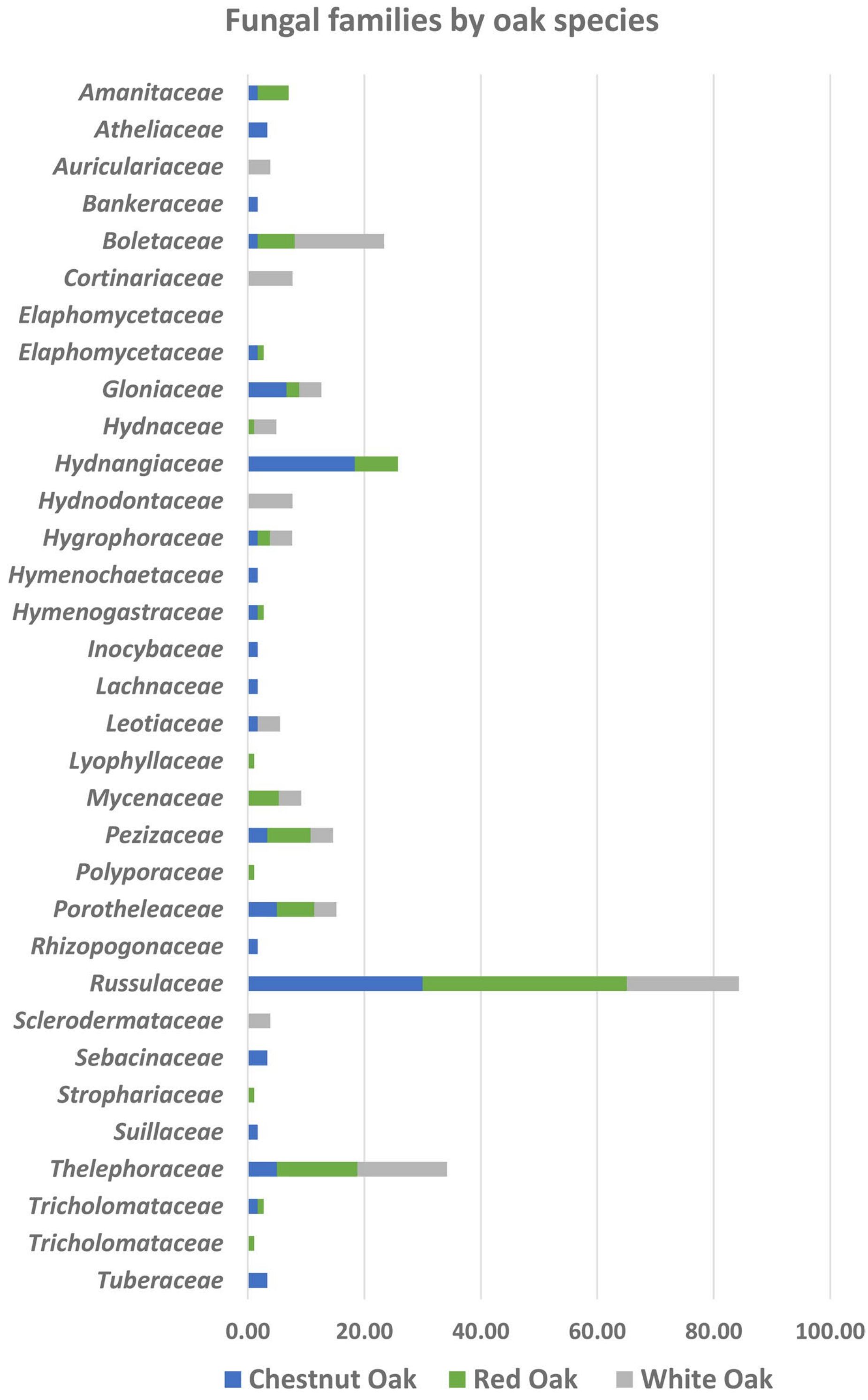

Fig. 7 Distribution of fungal families by oak group. Black oaks were combined with red oaks. Data were adjusted to a percentage to adjust for sampling size differences between red, white, and chestnut oaks. For example, 26.27% of ECM fungi on chestnut oak roots were in the *Russulaceae* (*Russula*, *Lactarius,* and *Lactifluus*), 32.04% of ECM fungi on red/black oak roots were in the *Russulaceae* and 27.59% of ECM fungi on white oak roots were in the *Russulaceae*

Table 3 Total richness and rarity of taxa from oak seedling root tips

Study	Total root tips	Successfully sequenced root tips	Total OTUs (Taxa)	OTUs with a frequency of one (rarity)
This study	361	236 (65.4%)	124 (52.1%)	96 (77.4%)
Walker et al. 2005	593	291 (49%)	75 (25.77%)	42 (56%)

natural and anthropogenic gradients. They demonstrated that mycorrhizal composition varied with soil pH, root density, and especially nitrogen pollution. Communities tended to have 2–3 dominant taxa and "many" rare taxa for a total of 392 taxa. García-Guzmán et al. (2017) carried out a meta-analysis of 14 oak communities in Europe, Western North America, and South America. Eastern North America was not included. They identified 1065 fungal taxa, 812 of which were represented only once. Thus, fungal populations can be patchy and/or influenced by the ecosystem, leading to an increase in estimated mycorrhizal richness with an increase in area and environmental conditions.

Taxonomic distribution of diversity

Among the taxonomically richest groups in the Walker et al. (2005) study were members of the *Tomentella-Thelephora* clade (19.72%), *Russulaceae* (*Russula* + *Lactarius*/*Lactifluus*; 25.35%), and *Cortinarius* (15.49%). Interestingly, only one of Walker et al. (2005) *Russula* OTUs was conspecific with an oak ECM in our data set despite geographically and ecologically similar ecosystems. Morris et al. (2008a, b) found that the ECM community of *Quercus crassifolia* Bonpl. in a montane cloud forest in Mexico was dominated by *Thelephora-Tomentella* (22.69%), *Russulaceae* (14%), and *Cortinarius* (7.33%). A survey of oak ECM fungi from the Cuitzeo basin in Mexico found the most species-rich family was *Thelephoraceae*, followed by *Russulaceae*, *Sebacinaceae*, and *Inocybaceae* (García-Guzmán et al. 2017). García-Guzmán et al. (2017) in a meta-analysis of oak ECM fungi from Western North America, Europe, China, and Mexico noted that these four families are also well represented in a number of other studies (Smith et al. 2007, Courty et al. 2008; Walker et al. 2008; Morris et al. 2009; Wang et al. 2012) but *Sebacinaceae* was hardly represented in this study. A study by Courty et al. (2008) of a temperate oak forest in France found that the ectomycorrhizal community was dominated by *Tomentellaceae*, *Russulaceae*, *Cortinariaceae*, and *Boletales*. Four species were abundant in the study site: *Lactarius quietus* (Fr.) Fr., *Tomentella sublilacina* (Ellis & Holw.) Wakef., *Cenococcum geophilum* Fr., and *Russula* Ru01. In the Walker et al. (2005) study, *Cenococcum* mycorrhizal root tips were ubiquitous but were not sampled for sequencing. This was true of other studies including this one. *Cenococcum* was frequently observed microscopically on roots but in this study, it usually failed to PCR amplify, perhaps an argument for morphotyping in addition to genotyping.

In all cited studies, multiple species of *Russula*, *Lactarius*, and *Thelephora-Tomentella* were recovered from oak root tips, but the findings of Walker et al. (2005) in the Southern Appalachian Mountains, together with the study by Courty et al. (2008) in France and Morris et al. (2008a, b), in Mexican cloud forests, contrast sharply with results of this study with respect to *Cortinarius*. While 11 types (OTUs) of *Cortinarius* were identified in the Walker et al. (2005) study and *Cortinarius* dominated in the Morris et al. (2008a, b) study, only one species, *Cortinarius olivaceopictus* Ammirati & A. H. Smith, was recovered in this study, one mycorrhizal root tip from a severely burned area and one from a moderately burned area. None of the cited studies showed the exceptionally high diversity of *Russula* OTUs that are present in this study. Finally, *Laccaria* is a dominant ECM species in the GSMNP but a relatively minor component of other studies. In this study, *Laccaria* aff. *laccata* was frequently recovered on ECM roots of oak. *Laccaria trichodermophora* is thought to be associated primarily with pine in the Southern Appalachians and with oak in Mexico. In this study, *L. trichodermophora* was clearly identified on an oak seedling root and from three roots associated with coppicing *Q. montana* so an association with oak in the GSMNP is also feasible.

In a separate partially overlapping study [see Hughes et al. (2020b) for experimental details], an Illumina MiSeq v3 analysis of soil samples taken from GSMNP paired burned and unburned sites recovered a higher proportion of *Russula* sequences in burned areas (unburned areas = 0.12%/32 samples; burned areas = 1.76%/59 samples) and higher species richness in burned sites persisting over a 2-year period. Thus, the proportionally higher proportion of *Russula* ECM on 1-year-old oak seedlings from burned and unburned soils may reflect both a higher proportion of *Russula* inoculum in the soil and a higher diversity of *Russula* OTUs in burned areas.

In the current study, three OTUs of *Russula*, *Russula* Ru10 (aff. *atropurpurea*), *Russula* Ru13, and *Russula* Ru17 (*R.* aff. *cyanoxantha*) were recovered in sufficient numbers to evaluate an association with burned or unburned soil. Of these, *Russula* Ru10 and Ru17 are mostly associated with unburned areas. *Russula* Ru08 (2 collections) and Ru13 (5 collections) are associated only with burned areas and may be heat-tolerant.

Cortinarius, a common ECM on oak, was hardly recovered from root tips in this study. *Cortinarius* is common and has been collected throughout the GSMNP. MyCoPortal lists 841 collections of *Cortinarius* (*and segregate genera*) from the GSMNP (MyCoPortal 2020). The possibility that *Cortinarius* may be sensitive to heat and thus was not present in high numbers in the soil after a wildfire is abrogated by the relative lack of *Cortinarius* ECM from unburned areas. Soils were sampled from some of the burned areas used in this study (Beals et al. 2022), and the taxonomic distribution of these soils was explored by sequencing for the ITS2 region using an Illumina MiSeq v3 [see Hughes et al., (2020b) for experimental details]. An exploration of this data set showed that *Cortinarius*, while present in soils, was rare in burned areas sampled for ECM in these studies. The majority of *Cortinarius* sequences in soil samples came from a single moderate burn site (Balsam Quiet Walkway), which was not sampled for the oak mycorrhizal study due to a lack of oak seedlings.

Effects of fire on diversity

Wildfires across terrestrial ecosystems affect species composition and community assembly including fungi (Bond and Keeley 2005). Numerous studies have concluded that extreme temperature generated by wildfires predominantly affecting the upper soil layers, loss of tree cover, decreased water infiltration, and altered physiochemical properties of the soil can cause drastic changes in the fungal community and possibly influence ecosystem function (Stendell et al. 1999; Dahlberg 2002; Trusty and Cripps 2011; Glassman et al. 2015; Dove and Hart 2017; Pérez-Izquierdo et al. 2020). Dove and Hart (2017) carried out a meta-analysis across studies, ecosystems, fire types, and methods of assaying species richness and colonization. They found an average reduction of 28% in fungal species richness. When mycorrhizal colonization was measured in situ, fire reduced mycorrhizal colonization by 21% (Dove and Hart 2017). Glassman et al. (2015) noted that a spore bank largely remains intact in severely burned *Pinus ponderosa* Dougl. stands (Sierra Nevada range, California), possibly providing inoculum for fungi after the fire.

In contrast to the above studies, we do not see an obvious or significant reduction in total fungal OTUs on roots from burned areas compared to unburned areas, but there are differences in OTUs between burn categories (Fig. 3) with the highest alpha diversity in areas with low/moderate burns based on several metrics. Low/moderate burns share some taxa with severely burned areas and some with unburned. Unburned and severely burned areas share the fewest taxa and highest beta diversity. Kivlin et al. (2021) found the arbuscular mycorrhizal fungi were not sensitive to fire severity in these same sites, and instead composition was driven by plant host association.

We speculate that the observed high alpha and beta diversity of ectomycorrhizal and root-associated fungi could be caused by an increase in fire-induced ECM patchiness in the soil which could result from local variation in heat intensity during a wildfire, variation in ECM heat tolerance, and/or sensitivity of both oak roots and fungi to altered post-fire soil composition (pH, carbon and nitrogen content, texture, moisture).

Prescribed fires vs. natural fires

Natural fires and prescribed fires in this study had a different fire history and so it is not surprising that they did not share many taxa, but distance and environmental factors could also explain the differences. The prescribed and natural fires are approximately 21 miles apart and have differing geologies, vegetation composition, and rainfall. In support of distance as an explanation, an examination of taxa in non-burn areas that were adjacent to the controlled burns and control areas for wildfire burns showed no overlapping taxa. However, communities under prescribed burns are more homogeneous according to the Betadisper analysis ($F = 6.0059$, $P = 0.01885$). Possibly, the repeated burns in the prescribed burn areas limited the availability of mycorrhizal fungi.

Oak decline and fire

Before European settlement, vast areas of the southern Appalachians were dominated by oak with *Quercus alba* (white oak) dominant and red oak comprising about 5% of the oaks (Abrams 2003). Red oak was limited by fire, drought, and greater susceptibility to disturbance, but land clearing, catastrophic fires, chestnut blight, and increased deer browsing decimated white oak populations while increasing populations of red oak and chestnut oak (Abrams 2003). Oak decline has been a concern in southern Appalachian forests for some years (Bechtold et al. 1987; Oak et al. 1995) and recently, oak recruitment overall has been limited on all but a few xeric upland sites (Abrams 2003). McEwan et al. (2011) suggested that changes in moisture and land use, but not fire exclusion, were controlling factors in oak decline.

Oak decline has been associated with changes in mycorrhizal fungal profiles and diversity (Kovacs et al. 2000; Pešková 2005; Lancellotti and Franceschini 2013) but not necessarily in number of mycorrhizal fungal root tips (Lancellotti and Franceschini 2013). This study suggests that mycorrhizal fungal availability is unlikely to be a limiting factor in oak recruitment, at least in the first year of growth but the dissimilarity in mycorrhizal fungal profiles between this study and several others suggests that fire may play an unexpected role in altering mycorrhizal fungal species on oak roots as they mature. Fire may have differential effects on non-symbiotic soil communities that can impact oak seedling success (Livne-Luzon et al. 2021). Beals et al. (2022) showed in many of these same sites that fungal pathogens were reduced after fire which allowed enhanced oak root growth, mediated by neighbor community identity. Thus, oak regeneration is likely due to a complex series of beneficial and non-beneficial interactions above- and belowground. Continuing observation during post-fire recovery might be warranted to further explore patterns of mycorrhizal fungal recruitment and may inform strategies for forest management going forward.

Conclusions

We hypothesized that we would find reduced mycorrhizal fungal species richness on oak roots in burned areas compared to unburned areas consistent with reports that wildfire reduces ECM spore bank richness (Tuininga and Dighton 2004; Buscardo et al. 2010; Glassman et al. 2015; Pérez-Izquierdo et al. 2020; Fox et al. 2022). We did not find that to be the case. Instead, there is increased species richness and diversity of ectomycorrhizal fungi in burned areas compared to controls and much of that diversity is in the families *Russulaceae*, *Thelephoraceae*, and *Hydnangiaceae*. The extensive colonization of *Laccaria* aff. *laccata* on oak roots in moderate/severely burned areas may suggest that *Laccaria* propagules survived the fire but a hypothesis of spore rain from adjacent unburned areas is also possible. The high alpha and beta diversity of OTUs within and between burn categories was unexpected. There was very little overlap in ECM fungal species from different burn categories. We also hypothesized that ectomycorrhizal and root-associated fungi from prescribed burns would differ from those recovered in the 2016 CT2 wildfire burn areas. The data support this observation but while differing fire regimes may play a role in the observed differences, there are a number of other factors (distance between sites, soil and rainfall, etc.) that also likely contribute to the observed OTU differences. It has been reported that fire increases overall diversity by creating "patchiness" within an ecosystem (Fox et al. 2022). This study is supportive of that observation.

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Author contribution KWH, ANM, and PBM conceived the overall project and carried out a parallel project identifying pyrophylous fungi. KWH supervised the oak project reported here and wrote the manuscript. KWH, AC, MA, and JF managed fieldwork. AC isolated and sequenced mycorrhiza, and JS selected and characterized field sites. SK helped with NextGen sequencing including data analysis. All authors edited the manuscript.

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Data availability The datasets used and/or analyzed during the current study are either included as supplementary materials or are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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