Forest Ecology and Management 503 (2022) 119774

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Belowground mechanisms for oak regeneration: Interactions among fire, soil microbes, and plant community alter oak seedling growth

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ARTICLE INFO

Keywords:
Oak regeneration
Fire
Soil
Soil microbial communities
Quercus
Pinus

ABSTRACT

It has been firmly established that oak regeneration benefits from prescribed burning and reduced competition with fire-intolerant tree species. Despite recommendations for research on the role of the microsite environment for oak regeneration, very little is known about the interacting effects of fire, soil, and surrounding plant community on oak establishment. We collected undisturbed and burned soil in the aftermath of a wildfire in Great Smoky Mountains National Park and used amplicon sequencing to identify differences in composition of bacterial and fungal communities between unburned and burned soils. To assess the effects of plant community, fire-induced shifts in soil microbial communities, and their interaction we conducted a glasshouse experiment and grew *Quercus velutina* seedlings in factorial treatments of plant neighbor (oak vs. pine seedling) and soil burn status (unburned vs. burned soil). Fire reduced the diversity of plant pathogenic and saprotrophic fungi and reduced the relative abundance of plant pathogenic fungi. Fire did not affect soil bacterial communities. Shifts in soil fungal community composition enhanced oak seedling root growth, but the effect of the soil microbiome was mediated by plant neighbor interactions. Seedling root growth was negatively correlated with diversity of pathogenic fungi. Root growth was enhanced in burned soil relative to unburned soil, but only when growing with a pine seedling neighbor as opposed to an oak seedling neighbor. Results from this study show that interactions between soil microbes and nearby plants can in part mediate oak seedling growth. As such, nuanced decisions that consider the ecological interactions of the microsite environment are needed to achieve desired outcomes for oak regeneration.

1. Introduction

Prescribed fire is the leading restoration tool for regenerating oak-dominated forests in the United States (Abrams, 1992; Brose et al., 2001; 2013) primarily because *Quercus* species have fire-adapted morphological and physiological traits (Reich & Hinckley, 1980; Abrams, 1992; 1990) and because frequent fire reduces other plant competitors that are less tolerant of fire (Wang et al., 2005; Nowacki & Abrams, 2008; Arthur et al., 2012). Prescribed burning can be implemented at different life stages of oak forest regeneration to target specific restoration outcomes (Arthur et al., 2012; Brose, 2014). For example, seedbed preparation burning is used to make the forest floor environment more hospitable for oak seedlings to establish, while release burning is used at a later growth stage to remove non-oak saplings and free young oaks from competition with mesophytic species (Brose, 2014).

While targeted prescribed burning methods such as these are beneficial, facilitating oak growth in the seedling development phase, in which oak seedlings are highly vulnerable to many environmental factors, likely requires more nuanced management practices targeted toward the forest floor environment. It is well-understood that high light availability, reduced herbivory, and reduced competition with other tree and shrub species is needed for successful oak seedling maturation (Arthur et al., 2012; Brose, 2014). What is less understood is the role of the soil environment, and specifically the interacting effects of fire, soil, and the plant community on oak seedling development, despite that recommendations for improvements to oak regeneration management have called for investigations on the role of the microsite environment (Arthur et al., 2012; Brose, 2014; Brose et al., 2013; Taylor & Midgley, 2018). For example, recent work by Taylor and Midgely on soil abiotic and biotic responses to prescribed burning in an oak-dominated forest concluded that "assessing the direct effects of burned soil on oak

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https://doi.org/10.1016/j.foreco.2021.119774
Received 19 August 2021; Received in revised form 5 October 2021; Accepted 7 October 2021
Available online 23 October 2021

regeneration is necessary to disentangle other burning effects, such as increased light availability, from soil effects on plant community composition" (Taylor & Midgley, 2018).

Soil microbial communities can be altered by burning, and these changes may be critical for oak seedling establishment. Fire can alter soil microbial communities through heat sterilization (Certini, 2005), changes to soil temperature and moisture (Treseder et al., 2004), changes to soil carbon and changes to inorganic nitrogen (Certini, 2005; Peay et al., 2009; Knelman et al., 2015). Fire generally reduces soil microbial diversity and abundance (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019; Sáenz de Miera et al., 2020), but soil fungi and bacteria often respond differently to fire. Some studies have documented greater decreases in soil fungi compared to bacteria (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019), in part because fungi are more sensitive to alkaline soils than bacteria (Bárcenas-Moreno et al., 2011; Rousk et al., 2010) and ash deposition from fire often increases soil pH (Certini, 2005; Peay et al., 2009; Knelman et al., 2015). Declines in mycorrhizal fungi may be greater than those of free-living soil fungi under certain scenarios, such as high severity fires, in which fire removes the respective plant hosts (Fultz et al., 2016).

Disentangling how the interactions between plant competitors and soil microbes affect oak seedling growth is crucial for improving oak regeneration after fire. The few studies that have examined the effects of fire on soil microbial communities in oak-dominated ecosystems have found mixed results. Wildfire reduced soil bacterial and fungal diversity in an oak-pine forest in western North Carolina (Huffman & Madritch, 2018), while prescribed burning in oak-dominated forests in Illinois resulted in increases in microbial biomass N but no changes in microbial biomass C (Taylor & Midgley, 2018). In contrast, prescribed burning conducted in an oak-dominated forest in Turkey yielded reduced microbial biomass C in burned plots (Akburak et al., 2018). Even less is known about how alterations to soil microbial communities from fire subsequently influence oak seedling development, and no research to our knowledge has examined this in conjunction with plant competition. Neighboring plants can influence how soil microbes interact with a focal plant. For instance, oaks frequently grow with pine species that are also fire dependent and some pine species are capable of transferring nutrients like nitrogen to nearby oak species through ectomycorrhizal networks (He et al., 2006).

The goal of this study was to identify the relative importance of fire-induced changes to soil microbial communities, intra- vs. interspecific plant competition, and the interaction of these two factors on oak seedling growth. We used amplicon sequencing to assess the diversity and composition of soil microbial communities collected from soil that had been burned by a wildfire in Great Smoky Mountains National Park (GSMNP) in 2016. Using this soil, we conducted a glasshouse experiment and grew black oak *Quercus velutina* seedlings in factorial treatments of soil inoculum (unburned vs. burned) and plant neighbor (black oak seedling vs. loblolly pine *Pinus taeda* seedling). Based on trends in the literature, we hypothesized that burned soils would harbor reduced diversity and abundance of bacteria and fungi relative to unburned soils (Hypothesis 1). We then hypothesized that reductions in soil microbial diversity and/or abundance caused by fire would influence oak seedling growth in one of two outcomes: either low microbial diversity and/or abundance in burned soil would enhance seedling growth or it would inhibit seedling growth (Hypothesis 2). Lastly, we hypothesized that oak seedling growth response to fire-induced changes to the soil microbiome would be influenced by the nearby plant community (Hypothesis 3). Based on personal observations that severely burned sites in our study system contained very few oak seedlings but many pine seedlings, we suspected that oak seedlings were unable to successfully colonize burned sites because they were outcompeted by pine seedlings. From these hypotheses and personal field observations, we predicted two possible outcomes in our glasshouse experiment (Fig. 1). If low microbial diversity and/or abundance of burned soil inhibits oak seedling growth, we expected oak seedling growth to be greatest when grown in unburned soil with an oak neighbor, followed by burned soil with an oak

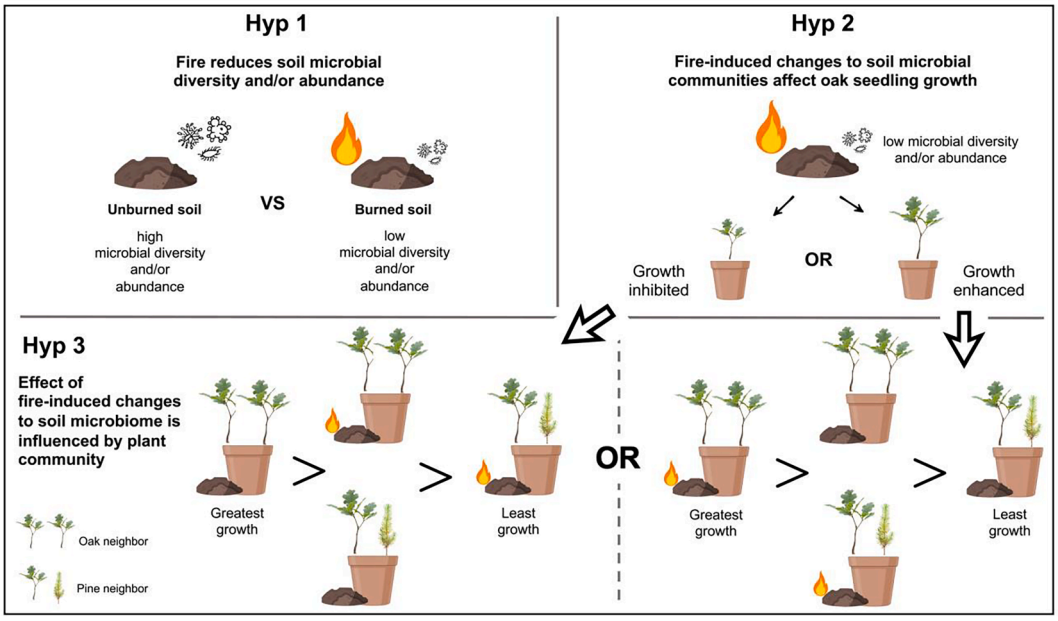

Fig. 1. The objective of this study was to identify the relative importance of fire-induced changes to soil microbial communities, type of plant competition, and the interaction of these two factors on oak seedling growth. Hypothesis 1) burned soils contain reduced bacterial and fungal diversity and/or abundance relative to unburned soils. Hypothesis 2) reductions in soil microbial diversity and/or abundance caused by fire either inhibit or enhance oak seedling growth. Hypothesis 3) oak seedling growth response to fire-induced changes to the soil microbiome is influenced by the plant community. If low microbial diversity and/or abundance of burned soil inhibits oak seedling growth, seedling growth would be greatest when grown in unburned soil with an oak neighbor, followed by burned soil with an oak neighbor or unburned soil with a pine neighbor, and growth would be lowest when grown in burned soil with a pine neighbor. If low microbial diversity and/or abundance of burned soil enhances oak seedling growth, seedling growth would be greatest when grown in burned soil with an oak neighbor, followed by unburned soil with an oak neighbor or burned soil with a pine neighbor, and growth would be lowest when grown in unburned soil with a pine neighbor.

neighbor or unburned soil with a pine neighbor, and growth to be lowest when grown in burned soil with a pine neighbor. If low microbial diversity and/or abundance of burned soil enhances oak seedling growth, we expected oak seedling growth to be greatest when grown in burned soil with an oak neighbor, followed by unburned soil with an oak neighbor or burned soil with a pine neighbor, and growth to be lowest when grown in unburned soil with a pine neighbor.

2. Methods

2.1. Study system

Field soil used in this study was collected from areas impacted by the Chimney Tops 2 (CT2) wildfire that occurred in the Great Smoky Mountains National Park (GSMNP) and in Gatlinburg, TN in November and December of 2016. The fire created a patchy gradient of burn severity within the fire perimeter in which some areas were unburned, some were low-moderately burned, and some areas were severely burned. To establish soil treatments representative of unburned and burned soils, we used a GIS fire severity raster map with a 30 m resolution generated by the US Forest Service Remote Sensing Application Center to randomly select candidate field sites that were severely burned and unburned. We chose to use severely burned sites to represent "burned" sites and we excluded low-moderately burned sites because they varied greatly in vegetation cover post-fire upon visual inspection. Soils in GSMNP are mostly loamy soils with a mixture of channery soil and fine sandy loam (Brown et al., 2019). We verified this by gathering soil texture classification from the Natural Resources Conservation Service (NRCS) Web Soil Survey database using the coordinates of each site (Table 1). We identified three 90 m^2 blocks within each burn status classification that were located within 300 m from roads and park trails for ease of accessibility for a total of seven sites (3 unburned sites and 4 burned sites). Because three of the burned sites were located in close proximity to one another, we included an additional burned site that was geographically distinct from the others. Sites were verified for differences in burn damage using the satellite-derived normalized difference vegetation index (NDVI) which quantifies the density of plant growth in a given area by measuring the intensity of light reflected by vegetation (Cihlar et al., 1991). To account for variation in canopy cover across sites before the Chimney Tops 2 fire, we calculated the delta NDVI (dNDVI) for each site, which is the difference in NDVI before and after the wildfire. We did this to accurately quantify only the difference in canopy cover due to damage from the wildfire. While we were not able to acquire ground measurements of burn, it has been shown that satellite-derived metrics of burn strongly correlate with ground measurements of burn (Cocke et al., 2005). Significant difference in dNDVI between the unburned and burned sites confirmed that the burned sites were in fact significantly damaged from the wildfire relative to the unburned sites ($\chi^2 = 36.3$, $p < 0.0001$) (Table 1).

2.2. Soil collection and processing

To assess changes to microbial communities in unburned and burned sites, we collected soil from each site in May 2019, approximately 2.5 years post-fire. Soils were collected by sampling from 10 random points within each site using a trowel to collect the top ~15 cm of mineral soil. Trowels were cleaned with ethanol between each site to reduce the possibility of microbial cross contamination. Following field collection, soil pH of each site was measured on air-dried soils in 0.01 M of $CaCl_2$ in the laboratory. An additional 2 g subsample of soil from each site was stored at −80 °C for assessment of microbial DNA. We used the Qiagen DNEasy PowerSoil DNA Isolation kit (Qiagen, Venlo, Netherlands) to extract DNA from 0.25 g of soil. DNA was eluted in 50 ul of buffer, and DNA concentration and quality were measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). Libraries were prepped using a two-step Polymerase Chain Reaction (PCR) approach with a mixture of 341F and 785R Illumina primers (Klindworth et al., 2013) to characterize bacterial 16S gene regions, and a mixture of 5.8S-FUN and ITS4-FUN primers to characterize fungal ITS2 gene regions (Taylor et al., 2016). Illumina-specific adapters were added to each forward and reverse primer to make them compatible with Illumina Nextera XT indexes. PCR for 16S rRNA and ITS2 were performed separately. The initial PCR consisted of 2x KAPA HiFi HotStart ReadyMix Taq (Roche, Indianapolis, Indiana, USA), 1.5 um of each forward and reverse primer per sample, and 2.5 ul DNA. A negative control of only nuclease-free water was included in the PCR and sequencing. The initial 16S PCR consisted of 3 min at 95 °C, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 sec, with a final extension at 72 °C for 5 min. The initial ITS2 PCR consisted of 2 min at 96 °C, followed by 30 cycles of 94 °C for 30 s, 58 °C for 40 s, 72 °C for 2 min, with a final extension at 72 °C for 10 min. Successful PCR amplification was confirmed by running 5 ul of PCR product alongside 5 ul of DNA ladder (GeneRuler 1 kb Plus DNA ladder 0.1 ug/ul, Thermo Scientific) on a 1.5% agarose gel. The PCR product was purified with AMPure XP beads (Agencourt, Beverly, Massachusetts, USA). Nextera XT indexes were then ligated to the PCR products with a second PCR of 95 °C for 3 min, followed by 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were purified again using AMPure XP beads. Samples were quantified on a NanoDrop 2000 spectrophotometer. Final PCR product size and concentration were confirmed on a Bioanalyzer. Amplicon sequencing was performed on the Illumina MiSeq platform at the University of Tennessee Genomics Core facility (Knoxville, TN, U.S.), using a 4 pM amplicon concentration including a 20% PhiX spike loaded onto a v3 600-cycle flow cell set for a paired-end read of 275 bases each.

All microbial genomic processing including primer removal was completed using a DADA2 pipeline (Callahan et al., 2016). Due to the variation in sequencing depth among samples, samples were normalized with a variance stabilizing transformation with the DESeq2 package (Love et al., 2014). We chose this method over the common practice of rarefaction because rarefaction results in loss of data by using the lowest sampling depth and it inflates variances across samples (McMurdie & Holmes, 2014). Taxonomy of amplicon sequence variants (ASVs) was assigned using the Ribosome Database Project (RDP) (Wang et al., 2007) and UNITE (Abarenkov et al., 2010) databases for bacteria and fungi, respectively. After processing the fungal sequences into ASVs, we

Table 1
Site characteristics of the seven field soil collection sites within Great Smoky Mountains NP. Soil texture classification was gathered from the NRCS Web Soil Survey database. Soil pH was measured in the laboratory. dNDVI represents delta normalized difference vegetation index which quantifies the density of plant growth in a given area by measuring the intensity of light reflected by vegetation.

Burn status	Site	Lat (dd)	Long (dd)	Elev (m)	Soil texture	Soil pH	dNDVI
Unburned	BGB	35.6809845	−83.5275497	517.09	fine sandy loam	5.09	0.0736
	PL441	35.7051697	−83.5240479	407.03	gravelly sand	5.67	0.0730
	RS441	35.7013168	−83.5252075	428.68	gravelly sand	5.44	0.0405
Burned	RGB	35.6829185	−83.5126953	628.82	sandy clay loam	4.34	0.2025
	FCM	35.6972008	−83.5328674	547.41	channery silt loam	4.20	0.2961
	SCM	35.6972008	−83.5328674	547.41	fine sandy loam	4.35	0.2961
	LCM	35.6972008	−83.5328674	547.41	fine sandy loam	4.23	0.2204

assigned ASVs to a fungal functional guild using the FUNGuild database of fungal taxa with known or suspected ecological functions (Nguyen et al., 2016). All fungal guilds were assigned at the genus level. We refined taxa to one of seven functional guilds: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, plant pathogenic fungi, saprotrophic fungi, and "other" (including animal pathogens, fungal parasites, epiphytes, lichen-forming, dung-inhabiting, etc.). For analysis we only included FUNGuild assignments with a confidence score of "probable" or "highly probable."

2.3. Glasshouse experiment

To address hypotheses that oak seedling growth is mediated by both fire-induced changes to the soil microbial communities, type of plant competition, and the interaction between these two factors (Hypotheses 2 and 3; Fig. 1), we conducted a reciprocal glasshouse experiment with factorial soil burn status × plant neighbor treatments. We chose to use *Q. velutina* and *P. taeda* for this study because both species occur in GSMNP at low-mid elevation (260–760 m. above sea level), which is similar elevation range to the sites included in this study and because both species had high seed availability from a commercial vendor. Acorns of *Q. velutina* and seeds of *Pinus taeda* were purchased from Sheffield's Seed Co. (Locke NY), and refrigerated at 4 °C prior to sowing. Acorns and seeds were sown into a commercial peat moss-based, non-mycorrhizal potting mix (Premier Promix BX, containing perlite, vermiculite, and limestone). Once emerged, oak and pine seedlings were transplanted into half gallon pots into eight treatments which consisted of factorial combinations of soil burn status (unburned soil, burned soil) and plant neighbor (oak or pine). Each pot was filled with a 5:1 ratio of Premier Promix BX potting mix:play sand and inoculated with 5.5 tablespoons of field soil (3% of total soil volume). We used a small amount of field soil to reduce any effects of variation in soil nutrients between inocula treatments, a common practice in soil inoculation experiments (Troelstra et al., 2001). Although the amount of field soil inoculum is very small relative to the volume of potting mix, it has been repeatedly shown that a small amount of microbial inoculum can induce plant responses (Lau & Lennon, 2011; 2012; Panke-Buisse et al., 2015). To confirm the effect of a field soil microbiome on plant function, we also included a control treatment in which pots contained only potting mix and no field soil. In total, 60 pots were established ([3 unburned field sites × 2 plant neighbor treatments × 3 replicates] + [4 burned field sites × 2 plant neighbor treatments × 3 replicates] + [2 plant neighbor treatments × 9 potting soil only controls]). To avoid positional effects, pots were randomly positioned in the glasshouse. Plants were equally watered from above, as needed (approximately 4 days/week), and allowed to grow for 4 months in a glasshouse at the University of Tennessee, Knoxville, TN, U.S.A.

A suite of plant phenotypes was measured throughout the duration of the experiment and post-experiment. Stem height was measured every two weeks and relative growth rates were calculated from these data. Prior to termination of the experiment, two terminal, mature leaves were randomly selected per oak plant, scanned using WinFOLIA software (Regent Instruments Inc.), oven-dried at 70 °C for 72 h (Pérez-Harguindeguy et al., 2016), and weighed to calculate specific leaf area (SLA; cm^2/g). In addition, we measured leaf chlorophyll content of one randomly selected mature top leaf for each oak using a CCM-300 chlorophyll content meter (Opti-Sciences). At 17 weeks, each individual was harvested and separated into above- and belowground biomass. Above- and belowground tissue was weighed after 48 h of oven-drying at 60 °C. Prior to drying, roots were carefully rinsed over 2 and 0.5 mm sieves to remove soil and collect all fine roots. We also measured average specific root length (SRL) for each individual using a digital scanner and WinRhizo software (Regent Instruments Inc.) prior to drying. Root to shoot ratio was calculated by dividing each individual's belowground biomass by aboveground biomass.

2.4. Statistical analyses

To test Hypothesis 1 (Fig. 1) that fire reduces soil microbial diversity, we calculated hill numbers based on ASV counts using the hill_div function in the hilldiv package (Alberdi & Gilbert, 2019). Hill numbers serve as effective numbers of diversity that provide more intuitive estimates of diversity compared to traditional diversity indices based on entropy (Chao et al., 2014). This method allows for separate estimates of raw richness and diversity that accounts for species evenness. We calculated hill numbers for all orders of diversity at $q = 0$, $q = 1$, and $q = 2$. A diversity order $q = 0$ provides raw richness by weighting rare taxa the same as abundant taxa. A diversity order $q = 1$ weights ASVs by their frequency without disproportionately favoring either rare or abundant taxa. A diversity order $q = 2$ overweighs abundant ASVs. For each order of q, we used the div_test function in the hilldiv package to test for differences in bacterial and fungal diversity between unburned and burned soils. We also assessed diversity within each fungal guild by further parsing the fungal ASV dataset into separate fungal guild datasets. We then calculated the relative abundance of each fungal guild among unburned and burned soils by dividing the sum of ASV counts of each fungal guild for each site by the total number of all ASV counts for each field site. We built linear models for each fungal guild using the lm function in the stats package in base R. We specified burn status as the fixed effect. We used the Anova function in the car package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares. When necessary, relative abundance was logit transformed to conform to normality prior to running models.

To test Hypotheses 2 and 3 (Fig. 1) that oak seedling growth is influenced by fire-induced changes to soil microbial communities, type of plant competition, and the interaction of these two factors, we used the lmer function in the lme4 package (Chao et al., 2014) to build linear mixed effects models with soil burn status, plant neighbor, and soil burn status × plant neighbor as fixed effects and soil inoculum field site as a random effect. For all analyses, separate models were built for each of the six oak seedling phenotypes (relative growth rate, shoot biomass, root biomass, SRL, SLA, leaf chlorophyll content). When necessary, phenotype data was transformed to conform to normality before analysis. For all models, we used the Anova function in the car package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares, with significance assessed for each fixed effect using Wald X^2 statistics. For phenotypes in which the effect of soil burn status was significant we then built separate linear mixed effects models to specifically test if differences in overall microbial diversity, fungal guild diversity, and relative abundance of fungal guilds influenced oak seedling phenotypes. For the diversity models, we included hill number at each order of diversity as the fixed effect and plant neighbor and soil inoculum field site as random effects. Separate models were built for each order of diversity. For the relative abundance models, we included fungal guild proportion (%) as the fixed effect and plant neighbor and soil inoculum field site as random effects. When significant, we calculated model fit (R^2) with the r.squaredGLMM function in the MuMIn package (Nakagawa & Schielzeth, 2013). If the effect of soil burn status × plant neighbor was significant, we conducted post hoc Tukey contrasts using the TukeyHSD function. All analyses were performed in R (R Team, 2020). Boxplots were made with the ggplot2 package (Wickham, 2016). Ordination figures were made with the phyloseq package (McMurdie & Holmes, 2013). Multi-panel figures were compiled with the patchwork package (Pedersen, 2020).

3. Results

Overall, we found support for two of our three hypotheses. Fire reduced species diversity of some components of the soil microbiome, and these microbial responses were associated with differential oak seedling growth, but only when oak seedlings were grown with a heterospecific seedling neighbor.

3.1. Sequencing output and fungal community composition

After processing, we had 2,912 ASVs of the total 134,465 bacterial sequencing reads and 1,657 ASVs of the total 190,297 fungal sequencing reads. Of the 1,657 fungal ASVs, 842 were assigned to a fungal guild, and the rest were unassigned. Of those assigned to a guild, we used the 616 ASVs that had a confidence ranking of "highly probable" or "probable." The majority of ASVs were assigned as saprotrophic fungi (65%), followed by "Other" (11.5%), ectomycorrhizal fungi (6.7%), arbuscular mycorrhizal fungi (6.2%), plant pathogenic fungi (6%), endophytic fungi (4.1%), and ericoid mycorrhizal fungi (0.5%). We excluded the ASVs assigned as ericoid mycorrhizal fungi and "Other" from further analysis because the dataset only contained 3 ericoid ASVs and the "Other" category was comprised of fungi that could not be grouped with any of the other six guilds and were thus not applicable to our system.

3.2. Soil microbiome response to wildfire

Soil microbial diversity was reduced in burned soils relative to unburned soils. However, this was only true for fungal communities and not for bacterial communities 2.5 years post-fire, providing partial support for Hypothesis 1. Unburned soils contained nearly three times as many fungal ASVs on average as burned soils at order of diversity q0 ($t = -5.79$, $p = 0.01$) (Table 2, Fig. 2b). Contrary to the majority of findings, burned sites in this study were lower in soil pH relative to unburned soils ($\chi^2 = 26.03$, $p < 0.0001$) (Table 1). As such, sensitivity of soil fungi to wildfire in these sites is likely due to other edaphic factors rather than soil pH. The overall fungal diversity did not significantly differ at orders of diversity q1 or q2 (Table 2, Fig. 2b), indicating that rare fungal taxa likely drove differences in diversity caused by fire. Bacterial diversity did not significantly differ between unburned and burned soils at any order of diversity (Table 2, Fig. 2a). Among the six fungal guilds, unburned and burned soils differed in the diversity of plant pathogenic and saprotrophic fungi. Unburned soil contained 2.4 times greater diversity of plant pathogenic fungi and 2.2 times greater diversity of saprotrophic fungi than burned soil at q0 (Table 2, Fig. 2c). Unburned soil also contained 2.6 and 2.4 times greater diversity of plant pathogenic fungi than burned soil at q1 and q2, respectively (Table 2, Fig. 2c). Diversity of saprotrophic fungi did not differ between unburned and burned soils at q1 or q2 (Table 2). Additionally, unburned soil harbored 2.5 times greater relative abundance of plant pathogenic fungi than burned soil (Table 3, Fig. 2d). The proportion of saprotrophic fungi was similar among unburned and burned soil (Table 3). Diversity and relative abundance of arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and endophytic fungi were similar among unburned and burned soils (Table 3).

3.3. Effect of fire-induced changes to soil microbial communities on oak seedling success

While oak seedling growth did respond to soil burn status, oak growth was enhanced in burned soil relative to unburned soil, contrary to our hypothesis (Hyp 2). Seedlings produced 25% more root biomass when grown in soils containing burned soil inoculum compared to soils containing unburned soil inoculum ($\chi^2 = 4.10$, $p = 0.04$; Table 5, Fig. 3a). Differences in overall fungal diversity caused by burn did not significantly affect oak seedling root biomass ($\chi^2 = 1.79$, $p = 0.18$). However, root biomass was significantly correlated with fire-induced changes to diversity of plant pathogenic fungi. Oak seedling root biomass was negatively correlated with plant pathogenic fungi at diversity q1 ($\chi^2 = 3.95$, $p = 0.05$; $R^2 = 0.11$) and q2 ($\chi^2 = 5.64$, $p = 0.02$; $R^2 = 0.13$; Table 4; Fig. 3c, d), but not correlated with relative abundance of plant pathogenic fungi (Table 4; Fig. 3b). Diversity q0 of saprotrophic fungi was not correlated with oak seedling root biomass (Table 4). Shoot biomass, relative growth rate, specific root length, specific leaf area, and leaf chlorophyll content were not affected by soil burn status (Table 5).

3.4. Interactive effect of soil microbiome and plant neighbor on oak seedling success

In support of Hypothesis 3, soil burn status and plant neighbor had a slight interactive effect on oak seedling growth ($\chi^2 = 3.43$, $p = 0.06$; Table 5). The benefit of a burned-associated soil microbiome on oak root biomass was only present when oak seedlings were growing with pines. Oak seedlings grown in soils containing burned soil inoculum produced 39% more roots when growing with pine seedlings compared to growing with other oak seedlings (Fig. 4c). The individual effect of plant neighbor was also significant. Across all soil inocula treatments, seedlings produced vegetative biomass more than twice as quickly ($\chi^2 = 5.63$, $p = 0.02$; Table 5, Fig. 4a) and produced 20% more root biomass ($\chi^2 = 7.06$, $p = 0.008$; Table 5, Fig. 4b) when grown with a pine seedling compared to another oak seedling. The significant interactive effect on root biomass shows that belowground growth was driven by the combination of a pine neighbor and fire-induced changes to the soil fungal community (Fig. 4c).

4. Discussion

4.1. Fire alters community composition of soil fungi rather than soil bacteria

We found that fire reduced soil fungal diversity, and that bacterial diversity was largely unaffected by fire. Since burned sites in this study were more acidic than unburned sites, it is likely that reductions in soil fungal diversity in this system are not due to soil pH. Although we

Table 2
T-test of the effect of soil burn status (unburned vs. burned) on ASV diversity of soil bacteria, fungi overall, and specific fungal trophic guilds. Diversity was calculated using hill numbers which represent effective numbers of diversity. A diversity order q = 0 provides raw richness by weighting rare taxa the same as abundant taxa. A diversity order q = 1 weights ASVs by their frequency without disproportionately favoring either rare or abundant taxa. A diversity order q = 2 overweighs abundant ASVs. Presented as t statistics, p-values, and directionality of response (where significant). Statistically significant results are shown in bold. AMF = arbuscular mycorrhizal fungi; ECM = ectomycorrhizal fungi.

	q0		q1		q2		
Microbial group	**t value**	**p-value**	**t value**	**p-value**	**t value**	**p-value**	**Directionality**
Bacteria	2.26	0.073	1.42	0.220	0.35	0.750	–
Fungi (overall)	**−5.79**	**0.011**	−3.08	0.077	−2.99	0.070	**Decrease with burn at q0 only**
AMF	−1.50	0.200	−1.34	0.240	−0.99	0.390	–
ECM	0.77	0.480	1.24	0.300	1.32	0.250	–
Endophyte	−2.40	0.063	−1.08	0.340	−0.95	0.400	–
Plant pathogen	**−5.47**	**0.003**	**−7.83**	**0.001**	**−6.48**	**0.002**	**Decrease with burn at q0, q1, q2**
Saprotroph	**−3.28**	**0.048**	−2.50	0.077	−1.50	0.220	**Decrease with burn at q0 only**

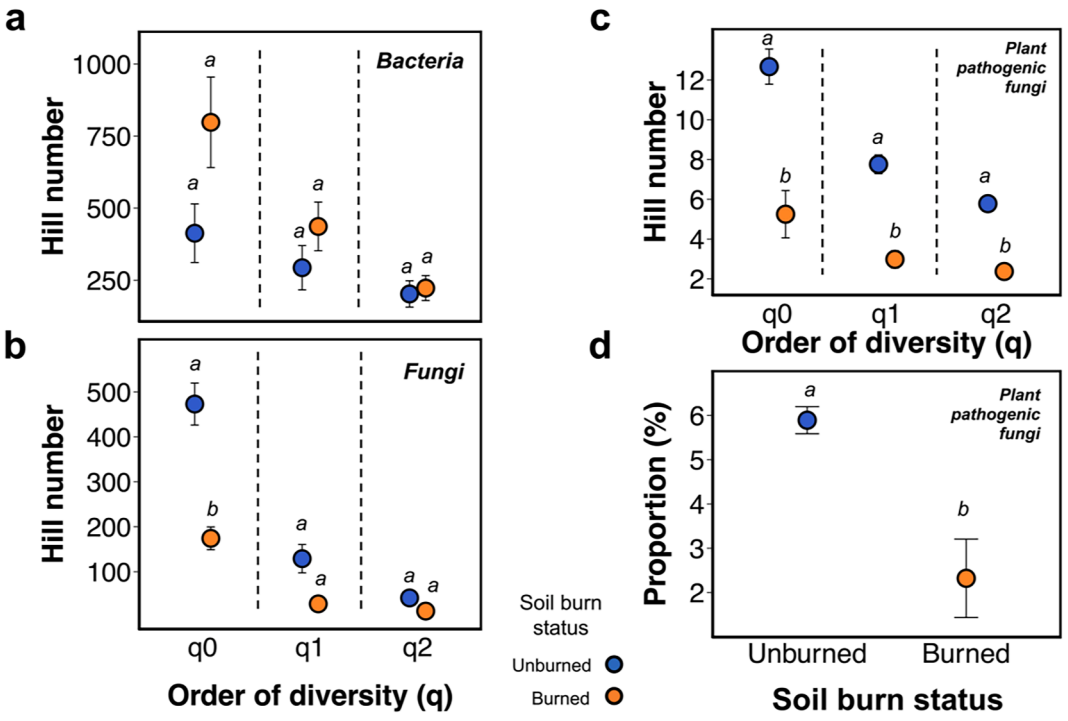

Fig. 2. ASV diversity of unburned and burned soils for bacteria (a), overall fungi (b), and plant pathogenic fungi (c) at various orders of q. Relative abundance of plant pathogenic fungi in unburned and burned soils (d). Data are pooled across field sites. Data points that do not share letters are significantly different from each other ($\alpha < 0.05$).

Table 3
One-way ANOVA of the effect of soil burn status (unburned vs. burned) on relative abundance of each fungal trophic guild. Presented as F statistics, p-values, and directionality of response (where significant). Statistically significant results are shown in bold. AMF = arbuscular mycorrhizal fungi; ECM = ectomycorrhizal fungi.

Fungal trophic guild	F value	p-value	Directionality
AMF	4.74	0.082	–
ECM	1.03	0.356	–
Endophyte	0.08	0.790	–
Plant pathogen	**10.97**	**0.021**	**Decrease with burn**
Saprotroph	0.03	0.859	–

cannot pinpoint the specific causes of soil fungal sensitivity to wildfire in this system, soil heating and drying caused by fire can negatively impact soil fungal communities because fungi in general are more heat-sensitive than bacteria (Bárcenas-Moreno & Bååth, 2009). Our overall findings, however, of greater reductions in soil fungi relative to soil bacteria in response to fire are consistent with other studies (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019). In our study, differences in overall fungal diversity are likely driven by rare taxa because unburned and burned soils only differed in diversity at order q0, and not at q1 or q2. This suggests that unburned soil has a greater number of rare fungal species compared to burned soil. A previous study that examined response of soil microbial communities to the CT2 wildfire also found that fungal richness was reduced in severely burned sites relative to unburned sites, but did not find changes in fungal species evenness with fire (Brown et al., 2019). As soil microbiome diversity is primarily comprised of rare taxa (Elshahed et al., 2008) our finding of only significant differences in number of rare species is not uncommon.

More specifically, we found that fire reduced the diversity of plant pathogenic fungi. Burned soil had significantly fewer fungal pathogen taxa than unburned soil. This was true for both rare and abundant taxa as unburned and burned soils differed for all diversity orders of q. Fire also affected the proportion of plant pathogenic fungi as the relative abundance was 2.5-fold greater in unburned soil relative to burned soil. While Brown et al. (2019) did not find differences in the relative abundance of plant pathogens between unburned and burned sites, their findings reflect soil fungal responses within six months after the CT2 wildfire whereas here we show fungal responses 2.5 years after the wildfire. We found that fire also reduced the diversity of saprotrophic fungi, but only by reducing the number of rare taxa. However, fire did not affect the proportion of saprotrophic fungi as the relative abundance of saprotrophic fungi was similar among unburned and burned soils. Other studies have found reductions in saprotrophic fungi after fire (Kaye et al., 2005; Brown et al., 2019; Day et al., 2019). Our finding that fire reduced saprotrophic raw richness (q0), but not species' evenness (q1 and 2) is similar to a previous study that found a decline in saprotroph richness with increasing burn severity, but no effect of fire on saprotroph species evenness (Day et al., 2019). Removal of organic carbon in upper layers of soil after fire and subsequent losses of lignin-rich litter may cause reductions in saprotrophs (Kaye et al., 2005). Overall losses of litter may correspond to lower litter diversity and a subsequent decrease in saprotroph species diversity.

In our study, fire did not affect mycorrhizal communities, as the diversity and relative abundance of both arbuscular- and ectomycorrhizal fungi were similar among unburned and burned soils. Our findings contradict a previous study that found reductions in ectomycorrhizal fungi from the CT2 fire (Brown et al., 2019). It is important to note, however, that these differences again may be due to time since the fire as the soil used in our study was sampled 2.5 years after the CT2 fire whereas the previous study sampled soil less than six months after the fire. Although a recent meta-analysis found that fire overall reduces richness of mycorrhizal fungi (Dove & Hart, 2017), a lack of response to fire in mycorrhizal fungi has been documented elsewhere, in a Canadian boreal forest system (Whitman et al., 2019).

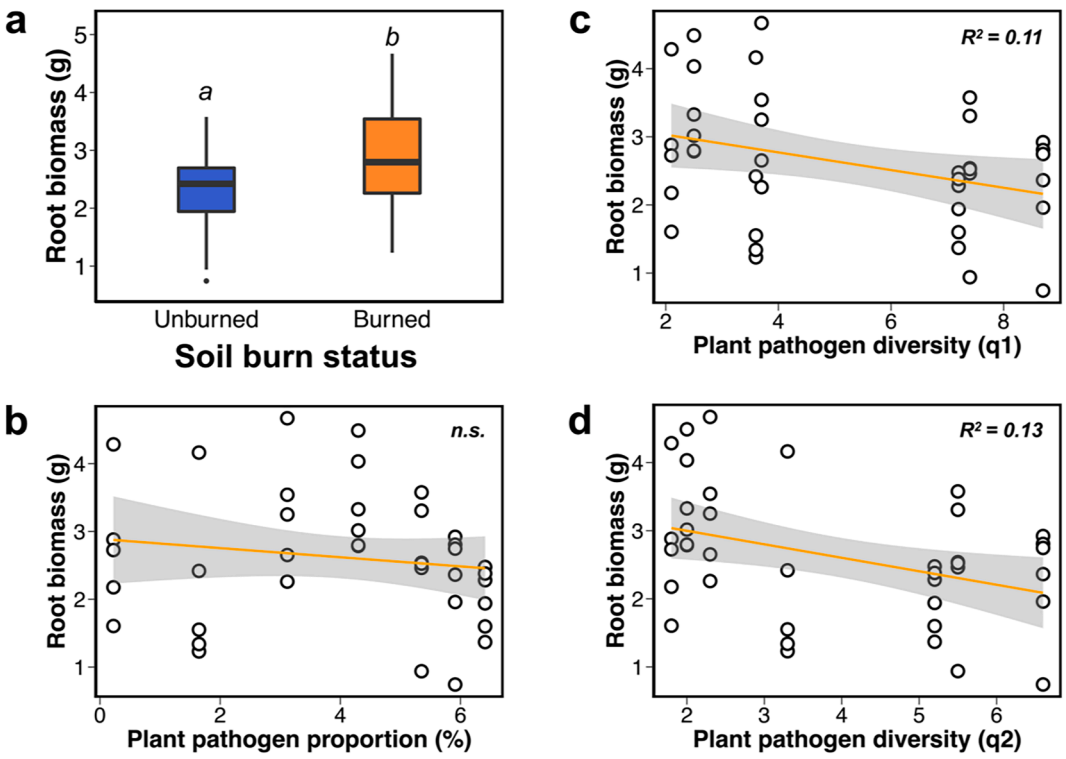

Fig. 3. Oak seedling root biomass when grown in treatments of unburned and burned soil inocula. Data are pooled across plant neighbor treatments (a). Data points that do not share letters are significantly different from each other ($\alpha < 0.05$). Correlation of relative abundance of plant pathogenic fungi (displayed as proportion) and oak seedling root biomass (b). Correlation of plant pathogenic fungal diversity (q1) and oak seedling root biomass (c). Correlation of plant pathogenic fungal diversity (q2) and oak seedling root biomass (d). Solid lines in figures b-d show predicted average values obtained using generalized linear mixed models with 95% confidence intervals represented by the shaded area. For all figures, root biomass data were pooled across plant neighbor treatments.

Table 4
One-way ANOVA of the effects of plant pathogenic fungi ASV diversity, plant pathogenic fungi relative abundance, and saprotrophic fungi ASV diversity on oak seedling root biomass. Presented as Chi-squared statistics, p-values, and directionality of root biomass response (where significant). Statistically significant results are shown in bold. Diversity was calculated using hill numbers which represent effective numbers of diversity.

	Root biomass			
Fungal trophic guild	**Effect**	χ^2	**p-value**	**Directionality**
Plant pathogen	Hill numbers (q0)	1.46	0.23	–
	Hill numbers (q1)	**3.95**	**0.05**	**Decrease with increasing diversity**
	Hill numbers (q2)	**5.64**	**0.02**	**Decrease with increasing diversity**
	Relative abundance	0.52	0.47	–
Saprotroph	Hill numbers (q0)	0.68	0.41	–
	Hill numbers (q1)	0.19	0.67	–
	Hill numbers (q2)	0.05	0.83	–

Table 5
Two-way ANOVA of the effects of soil burn status, plant neighbor, and their interaction on oak seedling phenotypes. Presented as Chi-squared statistics and p-values (p-values reported as 0.06 were rounded to the nearest hundredth decimal place). Statistically significant results are shown in bold. Shoot = aboveground biomass; Root = belowground biomass; RGR = relative growth rate; SRL = specific root length; SLA = specific leaf area; CHL = leaf chlorophyll content.

	Effect	χ^2	**p-value**
Shoot	Soil burn status	0.39	0.53
	Plant neighbor	2.59	0.11
	Soil burn × plant neighbor	0.63	0.43
Root	Soil burn status	**3.66**	**0.06**
	Plant neighbor	**6.57**	**0.01**
	Soil burn × plant neighbor	**3.43**	**0.06**
RGR	Soil burn status	0.24	0.62
	Plant neighbor	**5.66**	**0.02**
	Soil burn × plant neighbor	1.27	0.26
SRL	Soil burn status	0.32	0.57
	Plant neighbor	0.90	0.34
	Soil burn × plant neighbor	0.31	0.58
SLA	Soil burn status	1.46	0.23
	Plant neighbor	0.38	0.54
	Soil burn × plant neighbor	2.05	0.15
CHL	Soil burn status	1.62	0.20
	Plant neighbor	0.05	0.82
	Soil burn × plant neighbor	0.006	0.94

4.2. Fire-induced changes to soil fungal diversity alter oak seedling growth

In our glasshouse experiment we found that reductions in diversity of soil fungi caused by fire likely altered oak seedling growth. Seedlings grown in burned soil inocula produced significantly more root biomass than those grown in unburned soil inocula, and root biomass was negatively correlated with diversity of plant pathogenic fungi. Although saprotroph richness (q0) was reduced in burned soil and saprotrophs comprised over half of the fungal community compared to plant pathogenic fungi which only accounted for 6% of the fungal community, plant pathogen diversity was more important for oak seedling root growth than saprotroph diversity.

Specifically, increases in seedling root biomass in burned soil inocula relative to unburned soil inocula were likely driven by the abundant

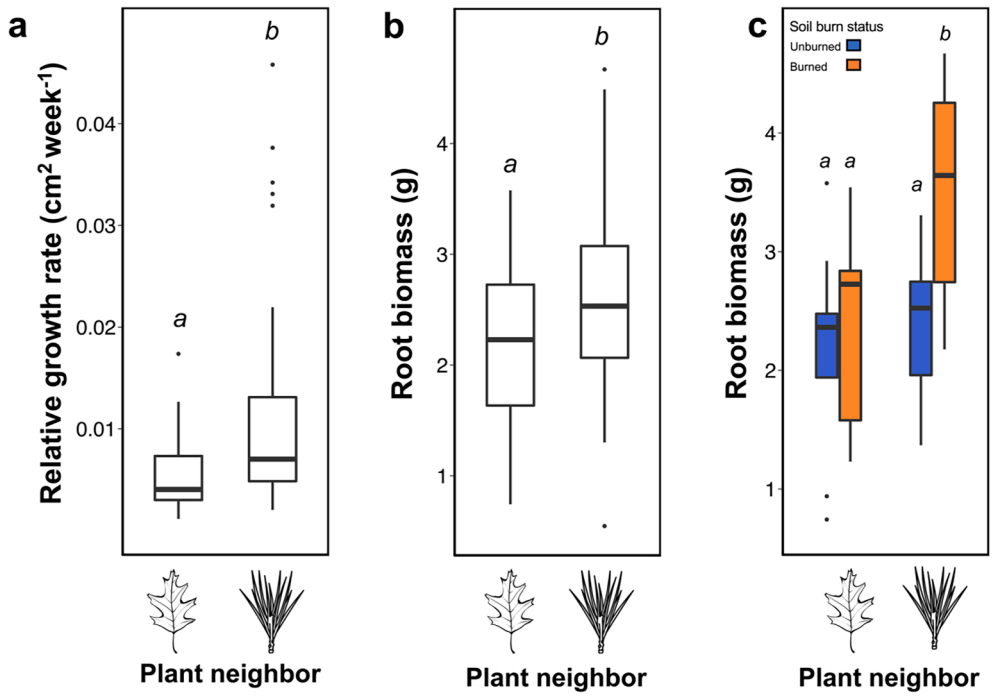

Fig. 4. (a) Oak seedling relative growth rate and (b) root biomass when grown in treatments of oak or pine seedling neighbor. Data are pooled across soil inoculum treatments. (c) Response of oak seedling root biomass to interactive effect of soil inoculum burn status and plant neighbor. Data points that do not share letters are significantly different from each other (Tukey test $p < 0.05$).

plant pathogen species rather than the rare species as significant correlations between plant pathogen diversity and root biomass were found at diversity orders q1 and q2 which account for species' evenness, but not at q0 which estimates raw richness. Plant pathogen relative abundance, however, was not correlated with seedling root biomass. These findings suggest that seedling root growth likely benefits from the lack of particular abundant plant pathogen species rather than the total number of plant pathogen individuals in the soil. These results are concurrent with the well-documented ecological phenomenon of enemy release from soil biota (Agrawal et al., 2005; Mangan et al., 2010; Comita et al., 2014).

Interestingly, a previous study of a similar experimental design found the near opposite outcome. Using soil and alder and spruce seeds collected from arctic tundra in Alaska, Hewitt et al. (2016) found a higher proportion of plant pathogenic fungi with increasing soil burn severity and found that tree seedling biomass declined when inoculated with burned soil inocula in a growth chamber experiment (Hewitt et al., 2016). While it is unclear why the abundance of plant pathogens is reduced by fire in one system and enhanced by fire in another, it is evident that tree seedling growth is inhibited in part by pathogenic fungi. Furthermore, our findings and those of Hewitt et al. (2016) show that fire can indirectly affect tree seedling success via soil microbial communities.

4.3. Fire and soil fungi effects on oak seedling growth influenced by plant species interactions

Our finding that oak seedlings produced more root biomass and produced aboveground biomass faster when growing with a pine seedling rather than another oak seedling contradicts our hypothesis (Hyp 2) that pine seedlings hinder oak seedling growth. This finding is, however, consistent with root resource partitioning. Belowground niche separation is a common mechanism for plant species coexistence. Vertical resource partitioning has been most notably demonstrated in savanna ecosystems with trees and grasses and is known as the two-layer hypothesis in which plant species with shallow root systems, such as grasses, take advantage of resources in the topsoil and plant species with deep roots such as trees primarily access resources in the subsoil (Ward et al., 2013). Oaks and pines also exhibit this ecological interaction, largely because of specialization in different water management strategies. Oaks are anisohydric and keep their stomata open which allows for high leaf gas exchange and the ability to photosynthesize under a wide range of moisture conditions (Poulos et al., 2020). Oaks also have a deep root system that compensates for this "riskier" water management strategy by allowing access to water in the deep soil layers (Williams & Snyder, 2003). Pines, in contrast, are isohydric and tightly regulate their stomata, which allows for drought avoidance, but an inability to photosynthesize and grow under low moisture conditions (Poulos et al., 2020). Pines have a shallow root system that allows access to water in the upper soil layers (Williams & Snyder, 2003). While we were not able to quantify and compare root niche space among oak and pine seedlings, we visually noticed stark differences in root structure between the oak and pine seedlings while harvesting plant tissue from the glasshouse experiment. All oak seedlings had a long taproot and pine seedlings had a much shallower root system. We did not measure soil moisture in the glasshouse experiment, but our findings likely indicate soil moisture partitioning as oak seedlings that were grown with a pine seedling neighbor were likely better able to mine the deeper soil layers compared to seedlings that were competing for the same soil space with another oak seedling. On the landscape, neighboring pine seedlings may benefit oak seedling establishment due to root niche partitioning. Other belowground interactions between oaks and pines may also facilitate oak success. For example, some research has found that some pine species are capable of transferring nutrients like nitrogen to nearby oak species through ectomycorrhizal networks (Poulos et al., 2020).

Lastly, we found that the effects of soil pathogenic fungi on oak seedling growth were mediated by the plant neighbor interactions. The soil enemy release advantage was only present when oak seedlings were growing with a pine neighbor. Similarly, the advantage of growing with a pine neighbor only occurred in soil containing burned soil inocula with reduced fungal pathogen diversity. Our finding contradicts our hypothesis (Hyp 3) that oak seedling growth would differ between all soil

burn status and plant community treatment combinations, when in fact treatments of oak neighbor-unburned soil inoculum, oak neighbor-burned soil inoculum, and pine neighbor-unburned soil inoculum all yielded similarly low root biomass compared to the combination of pine neighbor and burned soil inoculum.

These findings could be caused by multiple belowground interactions. It is possible that spatial separation of roots between seedlings somehow further enhances the benefit of the low-pathogen soil inoculum. Although our study did not test specific mechanisms like this, other research has found associations between root architecture and soil microbes. A study by Ulbrich et al. found that the relative abundance of certain fungal taxa was correlated to root length (Ulbrich et al., 2021). It is also possible that oak seedlings benefit from the combination of the low-pathogen inoculum and a pine-associated microbial community. It is well-established that soil microbial communities vary among plant species. As such, oak seedlings may benefit from pine-associated microbial communities that likely contain fewer oak-specific microbial pathogens. While our study is limited in mechanistic inference, other research has found benefits from the combination of interspecific plant competition and soil microbial communities. A recent study by Fitzpatrick et al. (2019), for example, documented an increase in plant fitness-related traits when a focal plant was undergoing interspecific competition and in contact with a soil microbiome.

4.4. Conclusions and management implications

This study shows that successful oak regeneration on the landscape may require understanding the effects of ground-level ecological interactions in addition to the direct impact of fire on oak seedling establishment and growth. Despite thoroughly documented evidence for the importance of soil microbes on plant growth and for the effect of fire on soil microbial communities, there has been limited research investigating the role of fire on plant-soil interactions (Senior et al., 2018). This is the first study, to our knowledge, to examine the individual and interactive effects of fire, soil microbial communities, and plant species interactions on oak restoration. Results from this study enhance our understanding of some of the belowground mechanisms for oak seedling success post-fire. Here we show that not all microbial groups respond equally to fire, with pathogenic and saprotrophic fungi showing the most sensitivity in these sites. We show that fire may alleviate oak seedlings from many interactions with soil enemies by reducing the abundance of particularly harmful pathogenic fungi. Moreover, this release from harmful soil biota is facilitated under root niche partitioning scenarios in which oak seedlings are growing in proximity with plants that have different root architectures.

Expanding on previous findings that prescribed burning in oak-dominated forests can alter soil nutrients (Taylor & Midgley, 2018), this study shows that fire effects on soil biotic conditions may also affect trajectories of oak regeneration. Additionally, our findings that interactions between soil microbes and the surrounding plant community can interact to alter oak seedling growth emphasize that nuanced decisions are needed to achieve desired outcomes for oak regeneration depending on the context of the microsite environment (Arthur et al., 2012; Brose, 2014; Brose et al., 2013; Taylor & Midgley, 2018). Specifically, oak seedling success may benefit in scenarios in which prescribed burning reduces the soil pathogen load or may be hindered if burning increases soil pathogens, as has been documented previously (Hewitt et al., 2016). While reducing competition with mesophytic plant species is important, oak seedlings may also benefit from growing in a heterogenous plant community that facilitates a diversity of rooting strategies. It is unknown how a glasshouse experiment like this one translates to the field. Further field studies are needed to directly test if these relationships are upheld on the landscape and to determine how site-specific environmental variation may influence interactions between soil microbial communities and plant neighbors and their subsequent effects on oak seedling growth. For example, while our study only examined the presence or absence of fire, soil microbial response will likely vary with burn severity, intensity, and frequency (Taylor & Midgley, 2018). This study underscores that fire is not a one-size-fits-all solution for oak regeneration. Accurate management of oak seedling establishment will also require consideration of the ecological interactions of the microsite environment, including those among soil microbial communities and the surrounding plant community.

Data Accessibility

All data and accompanying analysis code are available on GitHub at https://github.com/kbeals2/Oak-Fire-Soil-Microbes.

Declaration of Competing Interest

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

Acknowledgements

This research was supported by a grant awarded to J.A.S. and collaborators Karen Hughes and Jennifer Franklin (), as well as a University of Tennessee Summer Research grant awarded to A.E.S. and A.T.S. We thank GSMNP Research Coordinator Paul Super for granting permits. We thank Salley Reamer for assistance with soil processing. We thank Jeff Martin and Kaitlin Palla for assistance in the greenhouse at UTK. We thank staff of the UTK Genomics Core facility, with special thanks to Veronica Brown. We thank the anonymous reviewers who provided helpful comments to improve the manuscript.

Authors' contributions

J.A.S. designed the study. A.T.S. and K.K.B. conducted fieldwork. A.T.S. conducted lab benchwork. A.E.S. led the greenhouse experiment and data collection. K.K.B. analyzed the data and led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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