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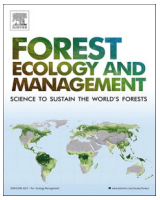

Fire-excluded and frequently burned longleaf pine forests have contrasting soil microbial communities

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ABSTRACT

Prescribed fires are common in forest management, yet we lack a clear picture of how different fire frequencies impact soil systems. Here, we present evidence of microbial community and soil chemistry shifts following sixty years of continuous prescribed fire interval manipulation at the Olustee Experimental Forest in Northeastern Florida. We investigated three fire interval treatments (1 year, 2 years, and 4 years) in addition to an unburned control treatment. We sampled three mineral soil horizons (A, E, and Bh) to elucidate prescribed fire impacts across the soil profile. Our results indicate that only the A horizon was affected by the fire interval manipulations, whereas the deeper E and Bh horizons were minimally impacted. Richness of both bacterial and fungal communities in recurring fire treatments was higher than, and their community composition different from, those in the unburned control in A horizon soils. Similar to the biotic soil attributes, fire interval treatments altered soil chemistry only in the top-most A horizon: the burned treatments had higher total nitrogen, total carbon, phosphorus, and NH_4^+ than the fire exclusion treatment; the soil chemistry of the deeper E and Bh horizons did not differ among the treatments. All soil chemistry properties correlated with bacterial community composition of the A horizon and nearly all properties correlated with fungal community composition of the A horizon as well, especially when comparing the more frequent burns to the fire exclusion treatment. Indicator taxon analyses identified fire-responsive bacteria and fungi, such as *Ktedonobacteria* sp. and an unclassified ascomycete that were abundant in the fire exclusion treatment and the ectomycorrhizal *Russula* spp. that were most abundant in the annual burn treatment. The different fire intervals also impacted fungal guilds, suggesting shifts in community function. The fire exclusion treatment was enriched with ectomycorrhizal, lichenized, and wood saprotrophic fungi, whereas the annual burn treatment was enriched with arbuscular mycorrhizal fungi compared to the other treatments. Our results indicate that long-term changes in the type and amount of detrital inputs and changes in the plant community associated with differing fire frequencies can induce shifts in the soil microbial community.

1. Introduction

Prescribed fires are a common forest management practice implemented to reduce fuel loads and to restore fire-adapted landscapes (Ryan et al., 2013). Fire suppression in fire-adapted landscapes can lead to shifts in resident communities, such as plant (Bond et al., 2004), animal and microbial (Pressler et al., 2019; Certini et al., 2021), biogeochemical cycling (Certini, 2005), and overall biodiversity (Mitchell et al., 2006). Prescribed fires have been widely used for centuries in the United States, and such fires are typically less severe than wildfires. Traditional ecological knowledge of prescribed burning is still used by Native Americans to promote certain plants, as some require fire to germinate (Agee, 1996; Anderson, 2005). Currently however, sociopolitical issues (e.g. encroachment of agricultural and residential land) and inadequate funding often preclude fire restoration in the United States (Ryan et al., 2013), which has resulted in an estimated loss of ~ 25 % of burn area (both prescribed and wildland) from 1998 to 2015 (Andela et al., 2017). Over 70 % of the land that is burned using prescribed fire in the United States is located in the southern states where most forest land is dominated by fire adapted tree species (pines, oaks, and hickories) (Kolden, 2019).

Many prescribed fire studies have focused on responses in plant

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communities or soil chemistry (Certini, 2005). In contrast, studies focusing on soil-inhabiting bacteria, archaea and micro-eukaryote communities and their responses to prescribed fire are fewer (Certini et al., 2021; Fox et al., 2022). These organisms provide important ecosystem functions such as nutrient and carbon cycling and play critical roles in the processes of decomposition and carbon sequestration (Crowther et al., 2019). In the past few years, however, research focusing on soil microbial community fire responses has increased (Oliver et al., 2015a; Dove and Hart, 2017; Pressler et al., 2019; Certini et al., 2021). Thus far though, only a few studies have aimed to elucidate how fires impact microbial communities and the soil chemistry in different soil horizons (Yang et al., 2020a; Yang et al., 2020b; Qin and Liu, 2021; Nelson et al., 2022). Most studies commonly sample only the top 10 cm of the soil because most of the biotic activities occur there and fire impacts are most obvious there (Certini, 2005; Joergensen and Emmerling, 2006). As a result, the impacts of maintained prescribed fire regimes on deeper horizons in the soil profile have received little attention. There have been a few studies (Stone et al., 1993; Gonzalez et al., 2018) that have focused on the deep soil processes and storage of carbon in the deeper soils (depth greater than 15 cm), but these studies lack the biological component that is provided in this study. Thus, with our study, we aim to fill these gaps and gain further understanding of the impacts of long term prescribed fire intervals on biological and chemical properties across three different mineral soil horizons.

In this study, we exploited a unique 60-year experiment to gain further insight into how prescribed fires impact soil chemistry and bacterial and fungal communities. Our primary goals were to (1) understand how the different fire intervals impact soil chemistry and microbial communities and (2) investigate how soil chemistry and microbial communities in different soil horizons (A, E, and Bh) respond to long-maintained fire interval manipulations. We hypothesized that (1) frequent fire intervals would result in a decline in diversity and richness of the microbial community, such as in the annual and two-year burns, (2) the annual burn communities would be enriched with pyrophilic microbes, and (3) there would be a loss of C and N in the more frequent burns, especially in the A horizon.

2. Materials and methods

2.1. Study site

The experiment is located in the Olustee Experimental Forest within the Osceola National Forest in Baker County in Northeastern Florida (30°14′17″ N, 82°24′41″ W). This experimental forest is one of 19 maintained by the USDA Forest Service Southern Research Station. It was established in 1931, originally used to study naval stores (resin products used for water-proofing). The Olustee Experimental Forest has an annual temperature range of 7°C–40 °C, and has an average annual precipitation of 1520 mm. This ecoregion experiences the driest parts of the year from November to January (Adams et al., 2003). Overstory vegetation at the site is dominated by longleaf pine (*Pinus palustris* Mill). Understory vegetation at the site is characterized by shrubs such as *Quercus minima* (Sargent) Small (runner oak), *Serenoa repens* (Bartr.) Small (saw palmetto), *Ilex glabra* (L.) Gray (gallberry), and *Lyonia ferruginea* (Walter) Nutt. (fetterbush), along with a diverse array of grasses and herbs in the ground layer vegetation (Glitzenstein et al., 2003). Soils at the site are derived from marine sediments and classified as sandy, siliceous, thermic Ultic Alaquods (Sapelo series) (Watts, 1996; Soil Survey Staff, 2021).

2.2. Experimental design

We utilized a research infrastructure established in 1958 that has been continuously maintained by the partnership of the USDA Forest Service Southern Research Station and the fire managers of the Osceola National Forest since its initiation – for a total of sixty years at the time of our sampling in 2018. This region was originally maintained by wildfire and indigenous burning in intervals of approximately ten years or less prior to colonization (Glitzenstein et al., 1995). The objective of this broad long-term study was to evaluate long- and short-term vegetation responses to prescribed fire occurring at different return intervals (annual, every 2 years, every 4 years, and an unburned control); while some of these intervals are very short, the purpose of our study is to understand how frequent fires impact the system. We will use T1, T2, T4, and T60 for these treatments from here on. The experiment is arranged in six randomized blocks dispersed over a slight moisture gradient where exactly 1 plot for each treatment was assigned to each of the 6 blocks. Each block has one replicate of each of the four fire treatments. Each treatment in the experimental design was replicated in a 0.8 ha plot in each of the six blocks for a total of 24 plots across the six blocks and four fire interval treatments. All plots had been established in a longleaf pine stand that was approximately 50 years old at the time of establishment in 1958 in which experimental prescribed fires have been consistently conducted on schedule.

2.3. Soil sampling

We sampled soils in January 2018 within a week before the prescribed burns were to take place. Our sampling took place during a year when all burn treatments synchronized such that all prescribed burns were scheduled for burning and all burn intervals were as close to complete as possible. In other words, plots that were scheduled for annual burn cycles had been burned approximately 12 months ago, the two-year intervals had been burned approximately 24 months ago, and those scheduled for four-year intervals had been burned approximately 48 months ago.

To get a representative sample of the 0.8 ha plots, we selected seven representative dominant canopy trees (longleaf pine in all plots, with abundant mid-story hardwoods in many of the long-term unburned plots) avoiding edges of the plot. From each of these seven trees, 3 m south of the stem, we collected an auger core using a bucket auger, and sampled soils from the middle of the A, E and Bh horizons (Fig. S1) (Taylor et al., 2023). The depths of the horizons varied due to the differences in the water table at the site. The depth to the boundary between A and E horizons was an average of 12.11 ± 4.44 cm, and the depth to the boundary between E to Bh horizons was 44.47 ± 12.36 cm (Table S1). The O horizon was discarded, where present, because it was nearly absent in the annually burned treatment. Within each plot, the seven samples were composited into a single plot-level sample by soil horizon and homogenized manually for a total of 72 samples across the 24 plots and three sampled horizons. The homogenized, pooled samples were transferred into Ziploc bags, placed on dry ice and shipped overnight to Kansas State University for analyses. Upon arrival at Kansas State University, the soil samples were stored at 20 °C until further processing.

At processing, the soil samples were thawed and passed through a 2 mm sieve to remove roots and large fragments. Each sample was divided into three aliquots: 1) 10 g for DNA extractions and molecular analyses of soil-inhabiting bacterial and fungal communities, 2) 50 ml for soil chemistry analyses; and, 3) 10 g for gravimetric soil moisture analysis. The remaining soil was archived in 50 ml Falcon tubes at 20 °C. All samples were stored in a 20 °C freezer until further analyses.

2.4. Soil chemistry

Soil chemistry analyses were conducted by the Kansas State University Soils Testing Lab (Manhattan, Kansas). 50 ml Falcon tubes full of frozen soil (weights varied) were submitted for testing. These soils were prepared by drying overnight at 60 °C and then ground to pass through a 2 mm sieve. Subsamples of these soils were used for analyses of pH, total C, total N, Bray phosphorous, readily available inorganic N (NH_4^+ and NO_3^-), and soil organic matter (SOM). The following methods were used

to analyze the soils. Using a 1:1 (w:w) soil slurry of 10 g of soil and deionized water, the pH was measured using an automated system. Bray P, the potentially plant available P, was measured using HCl-ammonium fluoride extractant from 2 g of dried soil and a colorimetric assay. The inorganic forms of nitrogen (NH_4^+ and NO_3^-) were extracted from 2 g of dried soil using 1 M of KCl and Cd reduction for NO_3^- (Gelderman and Beegle, 1998) and run in separate channels in a flow analyzer to measure the ions simultaneously. Using 0.35 g of dried soil, total C and total N were measured using a LECO TruSpec CN combustion analyzer (LECO, St. Joseph, Missouri). Using a modified version of methods from Combs and Nathan (1998), SOM was measured using loss on ignition wherein 1 g of soil was dried at 150 °C for 2 h and ignited at 400 °C for 3 h.

2.5. DNA extraction, PCR amplification, and sequencing

DNA was extracted from all samples within six months of sampling. Environmental DNA was extracted from ~ 10 g subsamples using PowerMax Soil DNA Isolation Kit (MoBio, Carlsbad, California) following the manufacturer's instructions and stored at 20 °C until PCR amplification. The DNA was quantitated with an ND2000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and standardized to 0.4 ng/ul for PCR amplification of the bacterial hypervariable V4 region of the small subunit of the ribosomal RNA gene (16S) and the fungal Internal Transcribed Spacer (ITS2) region of the ribosomal RNA gene cluster. These targets were chosen because they are highly variable and optimal for the short paired-end Illumina MiSeq sequences. The amplicons were generated with 12 bp barcoded primers of the forward 515f and reverse 806r primers for bacterial 16S (Caporaso et al., 2012) and the forward primer fITS7 (Ihrmark et al., 2012) and reverse primer ITS4 (White et al., 1990) for fungal ITS2 region. All PCRs were performed in triplicate 50 μl reaction volumes with the following amounts: 5 μl dNTPs (200 μM), 5 μl each primer (1 μM each), 10 μl Phusion 5X HF Buffer with 7.5 mM $MgCl_2$, 10 μl DNA (4 ng), 14.5 μl molecular grade water, and 0.5 μl (1 unit) Phusion Green Hot Start II DNA polymerase (ThermoScientific, Pittsburg, Pennsylvania). Proofreading polymerase was chosen to minimize generation of amplification artifacts (Oliver et al., 2015a). Bacterial PCR reactions began with an initial denaturation for 30 s (98 °C), followed by 25 cycles of 98 °C for 10 s denaturing, 30 s of annealing (50 °C), 1 min extension (72 °C), and concluded with a 10-min final extension at (72 °C). Fungal PCR reactions began with an initial denaturation for 30 s (98 °C), followed by 30 cycles of 98 °C for 10 s denaturing, 54 °C for 30 s annealing, 72 °C for 1 min extension, followed by a final 10 min extension at 72 °C. We used *Saccharomyces cerevisiae* for a fungal positive control and *Escherichia coli* for a bacterial positive control. Molecular grade RNA- and DNA-free H_2O was used as a negative control. All PCR products were visualized in 1.5 % agarose gel to ensure the successful amplification of correct size DNA fragments.

A total of 30 μl of each of the three technical replicates were combined for a total of 90 μl of the PCR product for amplicon purification with the Mag-Bind RxnPure Plus Magnetic Bead Clean-up solution (Omega Bio-Tek, Norcross, Georgia) using a modified manufacturer protocol with a 1:1 ratio of PCR product to the magnetic bead solution and rinsed three times with 80 % EtOH. Following clean-up, a total of 200 ng for bacteria and 220 ng for fungi of DNA were pooled for sequencing. Illumina adapters and indices were added in four PCR cycles using KAPA Hyper Prep Kit (Roche, Pleasenton, California), and 0.5 μg starting DNA. The libraries were sequenced (2 × 300 cycles) using the Illumina MiSeq Personal Sequencing System at the Integrated Genomics Facility (Kansas State University, Manhattan, Kansas). The sequence data are available through the Sequence Read Archive under the Bioprojects: PRJNA847592 (fungi) and PRJNA847616 (bacteria).

2.6. Sequence analysis

Sequence data were processed using the bioinformatic program mothur (v.1.38.0; Schloss et al., 2009). For both bacteria and fungi, sequences were contiged, primer sequences trimmed, and any sequences with ambiguous bases or more than 8 homopolymers (fungi) and 7 homopolymers (bacteria) were filtered out. Fungal sequences were then truncated to the shortest sequence length in the data set (237 bp), and pre-clustered to minimize platform generated biases (Huse et al., 2010). Bacterial sequences were aligned using the SILVA (release 132; www.arb-sil- va.de) reference alignment followed by pre-clustering. Bacterial and fungal sequences were screened for chimeras using VSEARCH (Rognes et al., 2016), and sequences presumed chimeric were filtered out. Sequences were classified using mothur implemented Naïve Bayesian Classifier (Wang et al., 2007) against the UNITE database (v6; Kõljalg et al., 2013) for fungi and the RDP training set (v10) for bacteria. Non-target lineages were removed as well as any sequences that could not be classified. Operational taxonomic units (OTUs) were then clustered into OTUs using Vsearch for fungi and nearest neighbor algorithm for bacteria. We calculated Good's coverage, observed (S_{obs}) and extrapolated (Chao1) richness, Shannon's Diversity (H') and Shannon's evenness (E_H) using mothur in which we rarefied our data to 10,000 and 3,000 sequences per samples for bacteria and fungi, respectively.

2.7. Statistical analysis

Statistical analyses were conducted using R version 3.6.3 (R Core Team, 2020) and R studio version 1.1.143. All data were examined for normality and homogeneity of variance, and factors were logarithmically transformed as needed. We first analyzed our full datasets (microbial alpha - diversity parameters and soil chemistry) in a global Analysis of Variance (ANOVA) with fire interval and soil horizon as main effects in addition to their interaction to test if fire interval effects may vary depending on the soil horizon. We also tested for block effect, but found no evidence for blocking factor effects (Supplemental Tables 1 and 2) and omitted this term from final analyses. To better dissect the significant fire interval × soil horizon interaction terms, we divided our dataset by horizon to better focus on and understand the fire interval effects within each of the three sampled soil horizons. To do this, we used one-way ANOVA to test for the effects of fire interval separately for each soil horizon on bacterial and fungal richness, diversity, and evenness as well as on the soil chemistry variables that we measured. Where the ANOVA tests indicated differences among the treatments, we utilized Tukey's Honestly Significant Difference (HSD) post-hoc pairwise comparisons to identify which of the four fire intervals might underlie those differences.

To analyze and infer responses in the bacterial and fungal community composition to the fire interval treatments by horizon, we used the R package vegan (Oksanen et al., 2013). We derived Bray-Curtis distance matrices and compared dissimilarities among treatments using PERMANOVA, an adonis2() function (McArdle and Anderson, 2001) within the vegan package. These community data were visualized using Non-Metric Multidimensional Scaling (NMDS) ordinations using packages ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2020). To understand the differences in compositional responses amongst the microbial communities based on fire interval, we utilized the pairwiseAdonis packages (Martinez, 2020). To investigate the dispersion (community convergence or divergence) amongst the communities, we used betadisper() within the vegan package (Anderson and Ellingsen, 2006). We also fit our soil chemistry data to environmental vectors into our NMDS ordinations using the envfit() command in the vegan package. To investigate which OTUs were more abundant in one treatment compared to others, we utilized multiplatt() command of the indicspecies package (De Cáceres and Legendre, 2009; De Cáceres et al., 2010). Similar to the community analyses, we investigated the indicator OTUs by soil horizon.

To derive functional attributes of the fungal community members, we used FUNGuild (Nguyen et al., 2016) to assign our fungal OTUs to ecological guilds and trophic modes. FUNGuild is a python based

program that utilizes the FUNGuild database. With these data, we conducted one-way ANOVA and Tukey's HSD post-hoc tests to understand the how specific ecological guilds (arbuscular mycorrhizae (AM), ectomycorrhizae (EcM), lichenized fungi, and saprotrophs) differed among the fire intervals and among the soil horizons. We chose these specific fungal guilds because they may be particularly sensitive to fire (Holden and Treseder, 2013; Semenova-Nelson et al., 2019; Certini et al., 2021; Fox et al., 2022).

3. Results

3.1. Soil chemistry

Many soil chemistry responses to fire depended on the soil horizon as indicated by the fire interval × soil horizon interactions in our two-way ANOVAs for total N ($F_{6,61} = 4.85$; P 0.001), total C ($F_{6,61} = 5.29$; P 0.001), NH_4^+ ($F_{6,61} = 5.17$; P 0.001), and SOM ($F_{6,61} = 2.52$; P = 0.03). In contrast, soil pH ($F_{6,61} = 0.91$; P = 0.50), Bray-P ($F_{6,61} = 0.85$; P = 0.54), and NO_3^- ($F_{6,61} = 1.20$; P = 0.32) had no evidence of fire interval × soil horizon interactions (Table S4). All soil chemistry attribute responses to fire intervals were significantly different amongst the soil horizons from each other based on the soil horizon interaction from the two-way ANOVA (F > 3.58; P 0.03) (Table S3). These were characterized by fire interval responses in the A horizon which were not observed in the deeper horizons. In our one-way ANOVAs (Table S5; Fig. S2), in which we analyzed each horizon separately, total N ($F_{3,19} = 6.08$; P = 0.004), total C ($F_{3,19} = 6.50$; P = 0.003), Bray-P ($F_{3,19} = 18.37$; P 0.001), and NH_4^+ ($F_{3,19} = 5.87$; P = 0.005) differed among the treatments in the A horizon and were lower in the T60 treatment than in the other treatments (T1, T2, and T4) (Fig. 1; A-G). Further, pH varied among the treatments ($F_{3,19} = 5.14$; P = 0.009) and was lower in T1 and T60 than in T2 and T4 (Fig. 1; C).

3.2. Bacteria – Richness and diversity

The full bacterial dataset contained initially 12,212,699 sequences. After quality control and subsampling, we included a total of 6,680,050 reads in the analyses. Good's coverage (99.4 ± 0.48 %) indicated that the bacterial communities were sampled adequately. The bacterial communities were dominated by Acidobacteria (36.0 %), Verrucomicrobia (35.7 %), and Proteobacteria (26.6 %); followed by Actinobacteria (12.8 %), Planctomycetes (6.8 %), Chloroflexi (5.3 %), Firmicutes (2.6 %), Chlamydiae (1.3 %), and other less frequent phyla that were 1 % (totaling 2.6 %). A small proportion (~3.0 %) of the bacterial data remained unclassified beyond Domain Eubacteria.

Bacterial richness, diversity and evenness varied among the fire intervals and soil horizons. However, in contrast to soil chemistry, we observed no evidence for fire interval × soil horizon interaction for bacterial richness, diversity or evenness ($F_{6,60}$ 1.84; P > 0.11) in our two-way ANOVAs. Our by-horizon, one-way ANOVAs indicated that, within the A horizon, fire suppression led to lower bacterial richness and diversity: observed (S_{obs}) ($F_{3,19} = 15.23$; P 0.001) and extrapolated (Chao1) ($F_{3,19} = 10.43$; P 0.001) richness, as well as Shannon's diversity (H') ($F_{3,19} = 7.51$; P = 0.002) were lower in T60 than in the other fire interval treatments (Fig. 2; A-C). There was no evidence for fire interval treatment effects for evenness (E_H) ($F_{3,19} = 1.43$; P = 0.27) (Fig. 2; D). In contrast to the A horizon, in the E horizon, only bacterial evenness (E_H) differed ($F_{3,20} = 3.58$; P = 0.03) among the fire interval and was lower in T60 than in T1, whereas the intermediate fire intervals differed from neither (Fig. S3; H). In the Bh horizon, we observed no evidence for any fire interval effects on bacterial richness and diversity ($F_{3,19}$ 2.32; P > 0.11).

3.3. Bacteria – Community composition

We visualized the bacterial community data using NMDS ordinations (Fig. 3; A-C). Permutational analogs of analysis of variance (PERMANOVA) indicated that the bacterial community responses to fire intervals likely depended on the soil horizon as indicated by the nearly significant ($F_{6,60} = 1.54$; P = 0.054; $R^2 = 0.06$) fire interval × soil horizon interaction. Analyses by soil horizon indicated that the bacterial communities responded to fire interval in the A ($F_{3,19} = 2.52$; P = 0.002) and E ($F_{3,20} = 1.98$; P = 0.02) horizons, but not in the Bh ($F_{3,19} = 0.87$; P = 0.65) horizon. Pairwise PERMANOVAs indicated that bacterial communities in the shortest fire intervals (Table 1) differed from T60 and that T1 also differed from T4 in the A horizon (Table 1). In the E horizon, all fire intervals (T1-T4) differed from the fire exclusion (T60) treatment (Fig. 3; B). The pairwise dispersion tests provided no evidence for differences in bacterial community dispersion among the fire intervals in the A horizon ($F_{3,19} = 0.99$; P = 0.42), E ($F_{3,20} = 0.15$; P = 0.93), or Bh Horizon ($F_{3,19} = 0.86$; P = 0.48).

3.4. Bacteria - Indicator taxon

Our indicator taxon analyses identified OTUs underlying the observed community differences between the fire intervals and soil horizons. Like our community data, we analyzed the data by horizon to focus on how fire intervals may have impacted community members. In the A horizon, a total of 27 bacterial indicator OTUs were disproportionately more abundant in T1, three in T2, none in T4, and eight in T60 (Table S6). The most abundant indicators for T1 were OTUs representing unclassified bacteria (29.6 % of T1 indicators' abundance) and *Acidobacteria* (22.2 % of T1 indicators' abundance). Indicators for T1 are taxa that likely respond positively to fire or decline in abundance when fire intervals are longer. *Verrucomicrobia* represented 33.3 % of the total abundance of the indicators for T2 of the A horizon. The most abundant indicator for T60 treatment in the A horizon was OTU1918 (*Ktedonobacteria)* with a total 26 % of the abundance of indicators for the T60 treatment within the A horizon. Indicators for T60 are likely taxa that respond positively to fire exclusion or decline in abundance when any prescribed fire has been implemented in our experiment. In the E horizon, there were 11 indicator OTUs for T1, seven for T2, one for T4, and 45 for T60. In T1, *Firmicutes* accounted for nearly half of the indicator abundance, accounting for 46 %. Of the seven indicators in T2,

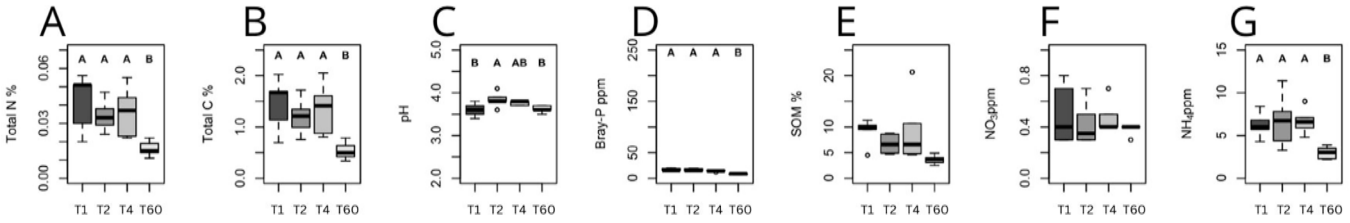

Fig. 1. Total nitrogen (%), total carbon (%), pH, Bray-Phosphorous (ppm), NO_3^- (ppm) and NH_4^+ (ppm) of the four different fire treatments (T1, T2, T4, T60) for the soil horizon A horizon-A:G).[All soil horizons in Fig. S2] Also included are the results of the Tukey's HSD Test as indicated by the letters above the boxes of the boxplot. Fire-interval treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = plots unburned for 60 years.

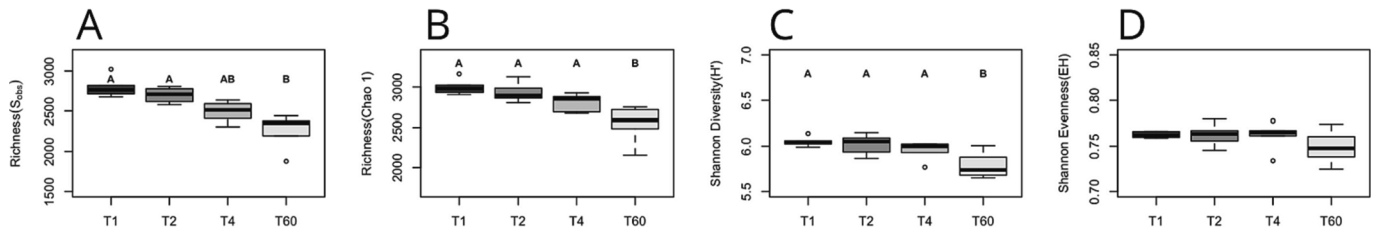

Fig. 2. Observed (S_{Obs}), extrapolated richness (Chao1), Shannon's diversity (H'), and Shannon's evenness (E_H) for soil bacterial communities within the four different fire treatments (T1, T2, T4, T60) for the A horizon-A:D [E and Bh horizon included in full figure in Supplemental S3]. Also included are the results of the Tukey's HSD Test as indicated by the letters above the boxes of the boxplot. Fire-interval treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = unburned for 60 years.

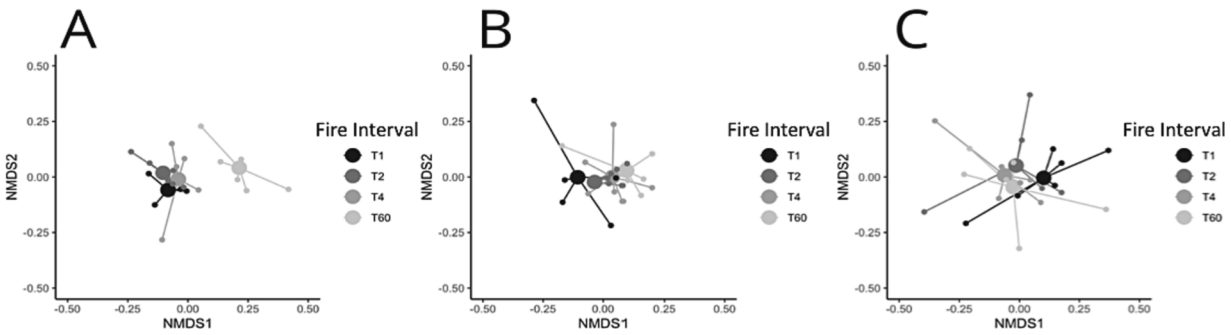

Fig. 3. Bacteria Non-Metric Multidimensional Scaling (NMDS) ordination spider plots of the four burn treatments (T1, T2, T4, T60) within the three soil horizons [A - A horizon (stress = 0.09), B - E horizon (stress = 0.07), C - Bh horizon (stress = 0.10)]. Legs indicate community dispersion from centroid (larger circle), smaller circles at the end of the legs are individual community samples. Fire-interval treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = unburned for 60 years.

Table 1
Pairwise permutational analysis of variance (PERMANOVA) results for bacterial community by horizon. Bold values indicate statistically significant (P 0.05) differences amongst burn intervals. Fire frequency treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = unburned for 60 years.

Pairwise Comparison	A Horizon			E Horizon			Bh Horizon		
	F	R^2	P	F	R^2	P	F	R^2	P
T1-T2	1.52	0.14	0.145	1.47	0.13	0.121	0.52	0.05	0.905
T1-T4	**3.21**	**0.26**	**0.016**	0.74	0.08	0.533	1.18	0.11	0.267
T1-T60	**3.61**	**0.26**	**0.007**	**3.62**	**0.39**	**0.022**	1.65	0.14	0.091
T2-T4	1.67	0.14	0.114	0.99	0.08	0.464	0.49	0.05	0.854
T2-T60	**3.27**	**0.25**	**0.003**	**2.04**	**0.16**	**0.047**	0.80	0.08	0.592
T4-T60	1.8	0.15	0.059	**3.12**	**0.24**	**0.012**	0.61	0.06	0.889

OTU3859, *Gammaproteobacteria,* was the most abundant indicator, accounting for 25 % of the indicators' abundance. The most abundant indicator for T60 in the E horizon was OTU450 (*Gammaproteobacteria*), 20 % of the indicators' abundance for the T60 treatment in the E horizon. In the Bh horizon, we identified a total of six indicator OTUs for T1, ten for T2, one for T4, and eighteen with T60. In the annual burn treatment (T1), *Alphaproteobacteria* (2 of the six indicators) and *Actinobacteria* (1 indicator) were the most abundant indicators with 7 counts each. OTU1098 (*Chlamydiae*) was the most abundant indicator for T2 accounting for 26 % of the abundance of indicators in the treatment. Similar to the A and E horizons, the indicators with the most OTU counts occurred in the T60 treatment. OTU2766, belonging to *Alphaproteobacteria*, was the most abundant indicator for T60 in the Bh horizon (Table S6).

3.5. Bacteria – Environmental correlates

Our analyses of the environmental correlates for bacterial communities indicated that all measured soil chemistry variables correlated (R^2 > 0.29, P 0.026) with the bacterial community composition (Fig. S5; Table S7) in the A horizon. The correlates with the highest coefficients were Bray-P (R^2 = 0.52; P = 0.002) and SOM (R^2 = 0.54; P = 0.002). Similarly, in the E horizon, all soil chemistry measurements correlated (R^2 > 0.25, P 0.038) with the bacterial communities except pH (R^2 = 0.21; P = 0.097). Soil NO_3^- (R^2 = 0.61; P = 0.004) and total C (R^2 = 0.47; P = 0.014) were the correlates with highest coefficients in the E horizon. The environmental correlates of the bacterial communities were fewer in the Bh horizon, where only pH (R^2 = 0.36; P = 0.017) and NH_4^+ (R^2 = 0.32; P = 0.023) correlated with the community data (Fig. S5; Table S7).

3.6. Fungi – Richness and diversity

The fungal dataset initially contained 3,225,289 sequences. After quality control and subsampling, the final sequence count was 1,510,145. Similar to bacteria, the fungal diversity was well represented in our sampling as indicated by Good's coverage (99.8 ± 0.51 %). The fungal data were strongly dominated by Basidiomycota (54.2 %) and Ascomycota (41.0 %) with the remaining percentage being less than 3 % for other fungal phyla and 2.5 % that could not be classified to phyla.

Similar to bacteria, we observed no evidence for fire interval × soil

horizon interaction in our two-way ANOVAs for fungal observed (S_{Obs} – $F_{6,60} = 1.67$; $P = 0.14$) or extrapolated (Chao1 –$F_{6,60} = 1.84$; $P = 0.11$) richness. In contrast, there was an interaction for diversity (H' – $F_{6,60} = 2.76$; $P = 0.019$) and evenness (E_H – $F_{6,60} = 2.94$; $P = 0.014$). In subsequent one-way ANOVAs, in which we analyzed each soil horizon separately, observed (S_{Obs} – $F_{3,19} = 7.62$; $P = 0.001$) and extrapolated richness (Chao1 – $F_{3,19} = 8.27$; $P = 0.001$) as well as Shannon's diversity (H' – $F_{3,19} = 11.69$; P 0.001) and evenness (E_H – $F_{3,19} = 9,47$; P 0.001) differed among the fire interval treatments in the A horizon (Fig. 4; A-D). Pairwise comparisons indicated that richness (S_{Obs}, Chao1) was lower in T60 than in any of the burn treatments (T1-T4) (Fig. 4; A and B). In the A horizon, diversity and evenness were lower in T1 and T60 than in T2 and T4 treatments. Similar to the bacterial analyses, there were no fire interval treatment effects on fungal richness and diversity in the E horizon (Fig. S4; E-H). Fungal observed (S_{Obs} – $F_{3,19} = 2.11$; $P = 0.133$) and extrapolated richness (Chao1 – $F_{3,19} = 2.12$; $P = 0.132$) did not differ among the fire intervals in the Bh horizon, whereas fungal diversity (H' – $F_{3,19} = 5.21$; $P = 0.008$) and evenness (E_H – $F_{3,19} = 4.77$; $P = 0.012$) in T1 and T2 were higher than in the T4 and T60 treatments in the Bh horizon (Fig. S4; K and L).

3.7. Fungi – Community composition

Fungal community responses to fire interval treatments differed among the soil horizons as indicated by the fire interval × soil horizon interaction (PERMANOVA: $F_{6,60} = 1.39$; $P = 0.002$; $R^2 = 0.09$). In subsequent analyses and in contrast to bacterial communities, fire interval treatments differed in each of the three soil horizons: A horizon ($F_{3,19} = 3.05$; $P = 0.001$); E horizon ($F_{3,20} = 1.63$; $P = 0.001$); Bh horizon ($F_{3,19} = 1.61$; $P = 0.01$). Pairwise comparisons indicated that in the A horizon, all burn treatments (T1-T4) differed from the fire exclusion treatment (T60), and fungal communities in T2 and T4 were distinct from T1 (Table 2; Fig. 5; A). In the E horizon, T1 and T2 treatments, but not T4, differed from T60 and the T1 communities also differed from T4 communities (Fig. 5; B). In the Bh horizon, the T1 and T2 treatments differed from the T4 and T60 treatments (Fig. 5; C). Similar to the bacterial analyses, we observed no evidence for differences in community dispersion in the fungal communities among the four fire interval manipulations in any of the three soil horizons: A ($F_{3,19} = 1.13$; $P = 0.36$), E ($F_{3,19} = 1.77$; $P = 0.18$), or Bh horizon ($F_{3,19} = 0.35$; $P = 0.78$).

3.8. Fungi – Environmental correlates

We also analyzed the environmental correlates for fungal communities. In the A horizon, all soil chemistry measurements correlated with the fungal community composition except SOM and NO_3^- (Fig. S6, Table S8). Bray-P ($R^2 = 0.72$; $P = 0.001$) and soil pH ($R^2 = 0.49$; $P = 0.002$) were the correlates with highest coefficients in the A horizon reflecting their observed low values in the T60 treatment. In the E horizon, NO_3^- was the only fungal community correlate ($R^2 = 0.31$; $P = 0.046$) (Fig. S6; B). In the Bh horizon, only pH ($R^2 = 0.48$; $P = 0.002$) and total C ($R^2 = 0.27$; $P = 0.041$) correlated with fungal communities, even though neither differed among the fire interval treatments in this horizon (Fig. S6; C).

3.9. Fungi – Indicator taxon

We utilized the indicator taxon analyses to identify those fungi that may have been disproportionately more abundant in one treatment than in others (Table S9). Similar to our other analyses, we analyzed the indicators separately for each soil horizon. In the A horizon, we identified 63 indicators for T1, 24 for T2, 12 for T4, and 37 for T60. The most abundant indicators for the A horizon were (T1) OTU 13, *Russula,* which accounted for 64 % of the T1 indicators' abundance in the A horizon; (T2) OTU 134 *Humidicutis,* 62 % of T2 indicators' abundance; (T4) OTU 878 *Basidiodendron* sp., 23% of the OTU counts; and, (T60) OTU 1953, *Trechispora coharens*, 56% of the OTU counts. The E horizon had a total of twenty indicators for T1, three for T2, ten for T4 and 34 for T60. The most abundant indicator OTUs for the E horizon were (T1) OTU 13 *Russula*, accounting for 82 % of the indicators' abundance for T1 of the E horizon; (T2) unclassified fungi, 50 % of the indicators' abundance; (T4) *Herpotrichiellaceae* sp. 27 % of the inidicators' abundance; and, (T60) unclassified *Clavariaceae,* 25 % of the indicators' abundance for T60 of the E horizon. In the Bh horizon, there were a total of 51 indicators for T1, 13 for T2 and 2 for T4 and T60 each. The most abundant indicator taxa for B horizon were: (T1) OTU 13, *Russula*, 20 % of the indicators' abundance for T1 of the Bh horizon; (T2) unclassified *Pleosporales* 26 % of the indicators' abundance; (T4) unclassified Fungi for both indicators with a total of 156 counts; and, (T60) *Talaromyces* 52 % of the indicators' abundance for T60 of the Bh horizon. Notably, OTU 13 representing the EcM genus *Russula* was among the T1 treatment indicators in all three soil horizons. In the A and E horizons in the fire exclusion treatment, OTUs representing unclassified ascomycetes were most abundant.

3.10. Fungi – FUNGuild

To gain insight into fungal ecological roles and their responses to fire interval treatments across the soil profile, we utilized FUNGuild. Of the total of 2,624 fungal OTUs, a total of 1,484 (57 %) OTUs could not be assigned to a guild or trophic modes. Further, some OTUs were assigned to more than one guild. When this occurred, we used the one listed first. Of the 1,140 FUNGuild assigned OTUs, we chose to focus on the two most common trophic modes: saprotrophs and symbiotrophs (representing 735 OTUs in total). This refined dataset included twelve guilds (Figs. S7 and S8). Among those, we analyzed the five most abundant guilds – AM fungi, EcM fungi, lichenized fungi, undefined saprotrophs, and wood saprotrophs.

We utilized two-way ANOVAs with fire interval and soil horizon and their interaction included in the model for each of the five guilds. The wood saprotroph guild was the only guild with a fire interval × soil horizon interaction ($F_{6,60} = 5.49$; P 0.001) suggesting that only its response to fire intervals depended on the soil horizon. In contrast, AM ($F_{6,60} = 0.38$; $P = 0.886$) and EcM ($F_{6,60} = 1.07$; $P = 0.389$) fungi, as well

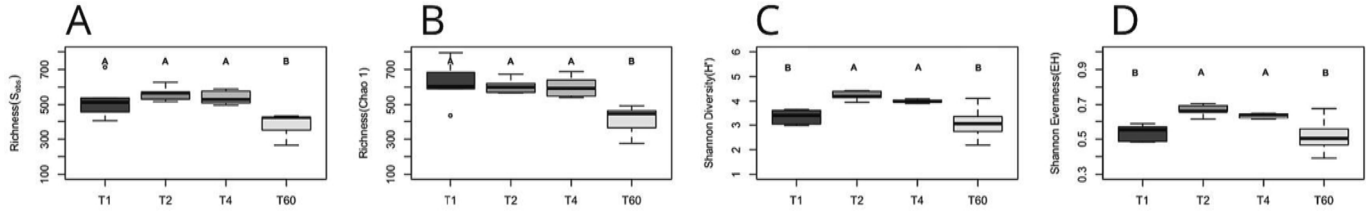

Fig. 4. Observed (S_{Obs}), extrapolated richness (Chao1), Shannon's diversity (H'), and Shannon's evenness (E_H) of fungal communities in four different fire treatments (T1, T2, T4, T60) for the 3 soil horizons (A horizon-A:D) [E and Bh horizon included in full figure in Supplemental S3]. Also included are the results of the Tukey's HSD Test as indicated by the letters above the boxes of the boxplot. Fire frequency treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = plots unburned for 60 years.

Table 2
Pairwise permutational analysis of variance (PERMANOVA) results for fungal community by horizon. Bold values indicate significant (P 0.05) differences amongst burn intervals. Fire frequency treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = plots unburned for 60 years.

Pairwise Comparison	A Horizon			E Horizon			Bh Horizon		
	F	R^2	P	F	R^2	P	F	R^2	P
T1-T2	**1.51**	**0.14**	**0.049**	1.25	0.11	0.097	1.39	0.13	0.061
T1-T4	**1.59**	**0.15**	**0.045**	**2.33**	**0.21**	**0.005**	**2.02**	**0.17**	**0.015**
T1-T60	**4.26**	**0.32**	**0.003**	**2.39**	**0.21**	**0.005**	**1.88**	**0.16**	**0.005**
T2-T4	1.31	0.12	0.117	1.25	0.10	0.127	**2.00**	**0.18**	**0.027**
T2-T60	**5.01**	**0.33**	**0.004**	**1.44**	**0.12**	**0.036**	1.66	0.15	0.041
T4-T60	**5.04**	**0.33**	**0.002**	1.25	0.11	0.214	0.75	0.70	0.675

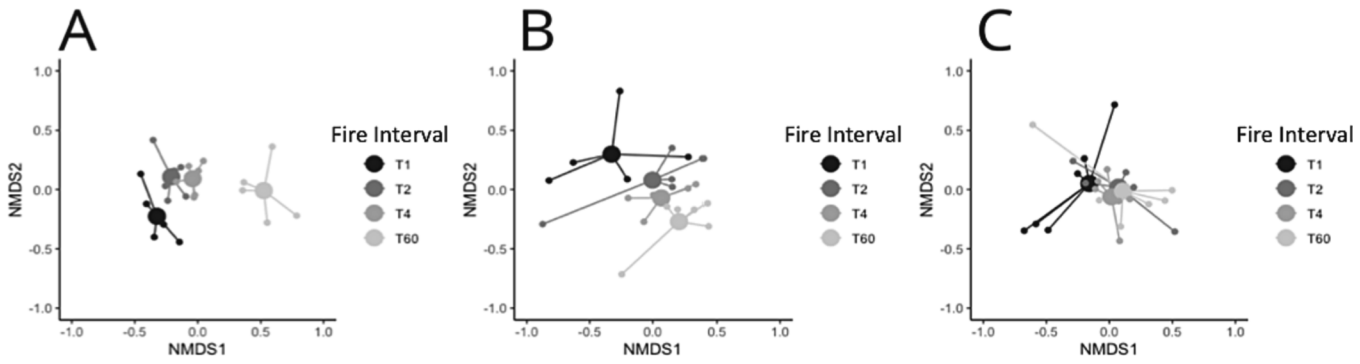

Fig. 5. Fungi Non-Metric Multidimensional Scaling (NMDS) ordination spider plots of the soil fungal community within the four burn treatments (T1, T2, T4, T60) and three soil horizons [A - A horizon (stress = 0.17), B - E horizon (stress = 0.18), C - Bh horizon (stress = 0.13)]. Legs of spider indicate community dispersion from centroid. Fire frequency treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = unburned for 60 years.

as lichenized fungi ($F_{6,60} = 1.40$; P = 0.230) and undefined saprotrophs ($F_{6,60} = 0.60$; P = 0.727) had no evidence for interactions. We then used one-way ANOVAs within each guild and soil horizon to gain further insight into how the fire intervals may have impacted guilds within each horizon (Fig. 6; A-D). Overall, four of the five guilds (AM fungi, EcM fungi, wood saprotrophs, and lichenized) analyzed in detail responded to burn interval treatments in the A horizon, whereas responses to burn intervals were fewer in the E and B horizons. Undefined saprotrophs did not respond to burn intervals in any horizon. In the A horizon, AM fungi differed among fire interval treatments ($F_{3,20} = 3.87$; P = 0.025). This was attributable to greater abundance of AM fungi in T1 than in T4 and T60 (T1 ≥ T2 ≥ T4 = T60). AM fungi also differed among the burn interval treatments in the E ($F_{3,20} = 5.12$; P = 0.009) and Bh ($F_{3,20} = 6.67$; P = 0.003) horizons. In these soil horizons, AM fungi were more abundant in T1 than in the other fire interval treatments. EcM fungi also differed among the fire interval treatments in the A horizon ($F_{3,20} = 4.40$; P = 0.02). In contrast to AM fungi, EcM fungi were more abundant in fire exclusion treatment (T60) than in T2 and T4 (T1 ≤ T60 > T2 = T4). EcM fungi did not differ among the fire interval treatments in the E ($F_{3,20} = 2.45$; P = 0.09) or Bh horizons ($F_{3,20} = 1.53$; P = 0.24). Lichenized fungi also differed in the A horizon ($F_{3,20} = 3.17$; P = 0.046), a result of their greater abundance in T60 than in T1 and T2. However, there was no strong evidence of responses in deeper E ($F_{3,20} = 2.85$; P = 0.063) or Bh horizons ($F_{3,20} = 0.88$; P = 0.466). Wood saprotrophs varied among the fire interval treatments in the A horizon ($F_{3,20} = 6.19$; 0.004), where their abundance was greater in T60 than in T1, T2, and T4. Wood saprotrophs also differed among the burn interval treatments in the E horizon ($F_{3,20} = 3.35$; P = 0.039), though only marginally in pairwise comparisons that suggested their greater abundance in T60 than in T2 and T4. In contrast to these four guilds, the undefined saprotrophs did not vary among the burn interval treatments in the A ($F_{3,20} = 0.68$; P = 0.571), E ($F_{3,20} = 1.66$; P = 0.207), or Bh ($F_{3,20} = 0.72$; P = 0.552) horizon.

4. Discussion

Our results indicate that prescribed fire intervals impact soil-inhabiting bacterial and fungal communities and soil chemistry, especially in the A horizon. In contrast, such responses deeper in the soil profile are subtler if not absent. In addition to fire responses that depended on soil horizon, our study highlights potential ecosystem context dependencies. For example, contrary to previous studies that report losses in soil C and N pools as a result of frequent fires (Neary et al., 1999; Holden and Treseder, 2013; Pellegrini et al., 2018; Mino et al., 2021), our results indicate that the more frequent prescribed burn treatments led to higher total C, total N, Bray-P, SOM, and NH_4^+ in mineral soil of the A horizon compared to the fire excluded treatment, contrasting our initial hypothesis. In a review on prescribed fire effects on soil properties, Alcañiz et al. (2018) concluded that there is no clear trend of soil chemical responses to fire. Rather, responses in soil chemistry depend on the system and, plant community composition, as well as fire regime and fire characteristics such as frequency, intensity, and severity (Pressler et al., 2019). It is important to note that the results of our study are only for the mineral soil horizons, and that we did not sample the O horizon, which (in long unburned systems) accounts for a large pool of organic matter (DiCosty et al., 2006) and presumably also microbial biomass. However, because the frequently burned plots had little to no O-horizon (particularly T1 and T2 treatments), we only sampled the horizons that all plots had in common, and evaluated these relative to the fire frequency treatments. Overall, our results corroborate and fall in line with other studies on soil chemistry differences in coastal pine systems and agree on differences between frequent fires and complete fire exclusion as well as on often minimal differences among the frequent burn intervals being mostly limited to the top layer of soils (McKee, 1982; Binkley et al.1992; Lavoie et al., 2010; Coates et al., 2018).

It is remarkable that after six decades of continuously maintained fire interval manipulations, very few measured attributes were distinct

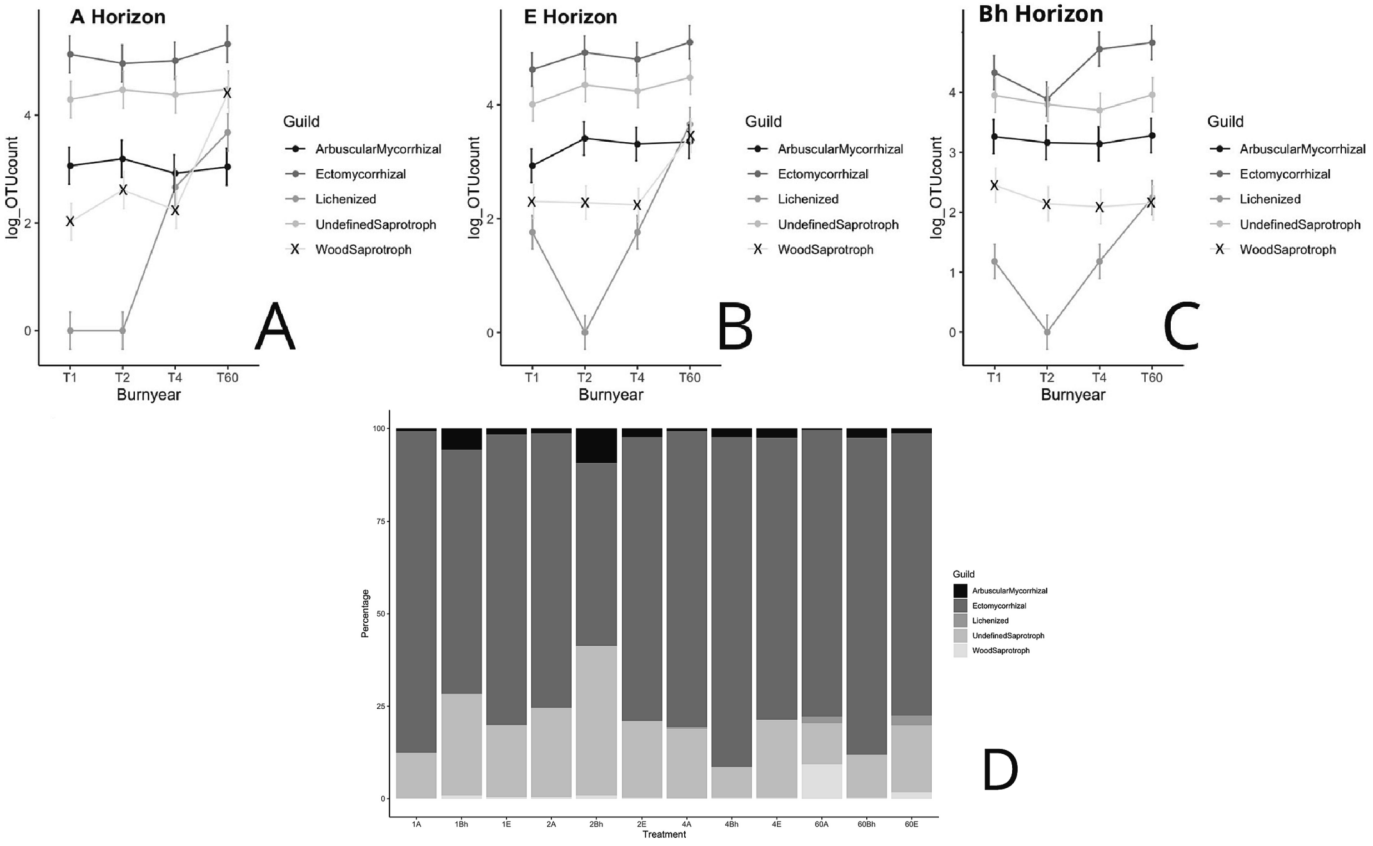

Fig. 6. Soil fungal functional guild abundance for each horizon based on fire interval (A, B, and C) with standard error bars. Soil fungal functional guild percentage based on fire frequency and soil horizon (D). Fire frequency treatment abbreviations: T1 = annually burned plots, T2 = plots burned every 2 years, T4 = plots burned every 4 years, and T60 = unburned for 60 years. For Panel D treatment labels, the numeral represents fire frequency and the alphabetic character represents soil horizon.

among the complete fire exclusion and annual prescribed burning in the deepest soil horizons (E and Bh). These observations highlight the rapid heat pulse attenuation within the soil profile (Massman, 2012; Smith et al., 2016; Bruns et al., 2020) and the resultant minimal impacts on soil chemistry and/or organisms deep in the soil profile that may be buffered from the short-term changes caused by fire. Long-term fire exclusion led to lower total C and N as well as lower Bray-P and NH_4^+ in the A horizon compared to the three treatments that included relatively frequent (up to every four years) prescribed burning. We also observed a decrease in total C and N (with a non-significant increase in the Bh horizon) and an increase in pH with increasing soil depth. Previous studies that have analyzed soil chemistry by horizon found similar results; importantly, carbon asserts a substantial control on microbial abundance and distribution in soil (Eilers et al., 2012; Jiao et al., 2018; and Xue et al., 2020). Our analyses of environmental correlates corroborate the importance of soil carbon for soil-inhabiting bacteria and fungi. Both SOM and total C correlated with bacterial communities in the two top-most soil horizons (A, E) and with fungal communities in the A horizon. It is of note, however, that in the A horizon nearly all measured soil chemical attributes correlated with the bacterial and fungal community composition. For example, total C, total N and Bray-P were strongly correlated with bacterial communities, and soil pH and Bray-P with fungal communities in the A horizon. Although soil pH is an important driver for bacterial communities in particular (Rousk et al., 2010; Xue et al., 2020), it was not among those with highest coefficients, perhaps emphasizing the substantial effects that recurring fires may have on top soils and their chemistry.

Our analyses did not support our hypothesis that microbial communities' diversity and richness would decline in the A with more frequently recurring prescribed fires compared fire exclusion treatment. Bacterial and fungal richness both responded to prescribed fire frequency similarly in the A horizon. Fire exclusion resulted in lower richness of both bacteria and fungi. Our results contrast Pérez-Valera et al. (2018) who observed higher microbial richness in the unburned sites, but this is likely attributable to our system being fire dependent forested system compared to a Mediterranean system (Kolden, 2019). Our results also contrast Mino et al. (2021), a fire manipulation experiment in a tallgrass prairie system that is fire dependent. These contrasting results could be due to differences in plant communities and/or biomass accumulation in the soil. However, our observed responses in richness were limited to the A horizon as there was no evidence for similar richness responses in either the E or Bh horizons. Microbial richness typically declines with soil depth (Jumpponen et al., 2010; Fierer et al., 2013; Santalahti et al., 2016). However, in contrast to other studies (Jumpponen et al., 2010; Santalahti et al., 2016), fungal richness and evenness were higher in the Bh horizon (relative to the shallower E), particularly in the treatments with most frequent fires (T1 and T2) (Fig. 4) – a pattern potentially attributable to the pedogenic processes that lead to spodic (Bh) horizon formation, specifically that organic matter is leached from the A and E horizons and then deposited at depth. Thus, the availability of organic matter for soil-dwelling organisms is greater at depth in Spodosols than may be typical of other soil types (Bacon et al., 2020).

Our analyses of community composition supported our hypothesis that fire exclusion would result in soil communities distinct from those observed in the long-term prescribed fire treatments. Bacterial communities were distinct among the fire interval treatments in the A and E horizon as the communities in the most frequently burned treatments (T1, T2) differed from the fire exclusion treatments. Interestingly, bacterial communities of the A horizon in the most frequent fire treatment

(T1) also differed from the less frequent fire treatment (T4). These observations suggest that even relatively small differences in maintained fire frequencies can have consequences for soil communities in the long term, and these are probably driven by changes in the plant community composition that are evident with changing fire frequencies (Glitzenstein et al., 2003). Similar to bacteria, fungal communities also responded compositionally to maintained fire interval treatments. In a similar pine ecosystem in Florida, Semenova-Nelson et al. (2019) also observed that burned and unburned fire treatments have distinct fungal communities. Overall, our results are consistent with others (Brown et al., 2013; Oliver et al., 2015b; Mino et al., 2021) who have reported that recurring prescribed fires alter fungal communities compositionally. However, although the fungal communities differed compositionally among the fire interval treatments in all three horizons, in the deeper E and Bh horizons only the shortest fire intervals (T1 and T2) maintained communities different from the fire exclusion treatment. These results support earlier speculation (Brown et al., 2013; Oliver et al., 2015b) that the longer fire intervals – here every four years – may permit system transition to conditions comparable to those without fire.

We utilized indicator taxon analyses to identify taxa that responded to different fire intervals. In general, we observed a number of potential indicators, particularly for the fire exclusion treatment, suggesting that some taxa disappear or become less frequent in systems experiencing greater fire frequencies. Among these indicators was a bacterial OTU assigned to *Ktedonobacteria* – the most abundant indicator for T60 in the A horizon. A chronosequence study of wildfire effects on bacteria in Canadian permafrost soils reported an increase of these bacteria in sites that were at least three years post fire (Zhou et al., 2020; Certini et al., 2021) suggesting it as an example of a bacterial taxon that is fairly intolerant of fire. In all three soil horizons, fungi assigned to the EcM genus *Russula* were the most abundant indicators for the most frequent fire treatment (T1). Several studies have indicated *Russula* spp. to be fire responsive, or more abundant following fire (Taudiere et al., 2017; Salo and Kouki, 2018; Rasmussen et al., 2018; Oliver, 2020; Dove et al., 2021), whereas others reported *Russula* spp. to decrease in abundance following fire, and are therefore fire sensitive (Dove et al., 2021; Pérez-Izquierdo et al., 2021). These results support our hypothesis that in the more frequent fire-interval treatments, we do indeed see enrichment of putative pyrophilic taxa. The differences in congeneric responses of *Russula* spp. may indicate ecosystem and context dependencies, or could potentially be species-specific responses to fire and conditions in the post-fire environments.

Although our indicator taxon analyses highlighted an EcM taxon as one more commonly responding positively to recurring fire, our functional guild analyses corroborated other studies (Castaño et al., 2020; Dove et al., 2021) that have concluded that EcM fungi overall are sensitive to fire and consequently decline in abundance after fire. Our guild-level analyses indeed indicated that EcM fungi were more abundant in the absence of recurring fire. In contrast to other studies (Day et al., 2019; Smith et al., 2020), we did not group all mycorrhizal fungi together, but rather analyzed EcM and AM fungi separately. Our data suggest contrasting responses of AM and EcM fungi. While EcM were most abundant in the fire exclusion treatment, the AM fungi were most abundant in the annual burn treatment (T1) in all three soil horizons that we analyzed here. Our results corroborate those of Treseder (2004), who also observed greater AM fungal abundance in recently burned sites than in the less frequently burned sites. These observations likely reflect changes in the plant communities upon which these fungi depend. The fire exclusion sites tend to include a greater number of EcM plants compared to the frequently burned sites that may include a greater proportion of AM hosts (Glitzenstein et al., 2003; Hart et al., 2005). In addition, some of our guild-level analyses provided intuitive results. For example, wood saprotrophs were most abundant in the fire exclusion treatment in the A horizon – an observation likely attributable to the more abundant woody substrates available in the absence of fire. Overall, these results support our hypothesis that there would be different community compositions based on fire frequency and/or exclusion and also highlights the potential functional shifts in fungal communities following fire.

5. Conclusions

Our use of this 60-year experiment allowed us to gain insight into how prescribed fires impact soils. Our study provided evidence that different fire intervals lead to changes in both soil chemistry and microbial communities. Importantly, these effects were most strongly visible in the topmost soils' A horizon. We cannot pinpoint the ultimate drivers that underlie these community changes or whether they are attributable to direct effects of the fire frequency (including fire exclusion) or subsequent changes in factors such as soil chemistry, particularly changes in soil carbon and organic matter that were often among the strongest correlates of the soil-inhabiting communities. Our study allowed us to demonstrate that prescribed fire regimes can impact the soil-inhabiting communities and that even relatively small differences in fire frequencies (annual vs. every four years) may have consequences on soil attributes. Importantly, our results showed that long-term fire exclusion has the greatest potential to change these communities, and our fungal guild analyses also strongly suggest that these community changes may also lead to changes in system wide functions as demonstrated by the declines in EcM fungi and increases in the AM fungi or wood decomposing fungi. This unique research opportunity enabled a great insight into how soils are impacted by long-term management practices. While most biological activity occurs in the top 10 cm of soils due to the abundance of roots and microbial activity (Eilers et al., 2012), having insight into the deeper horizons aids in our understanding of the long-term fire impacts.

CRediT authorship contribution statement

Sam Fox: Formal analysis, Visualization, Writing – original draft. **Melanie K. Taylor:** Methodology, Investigation, Writing – review & editing. **Mac Callaham Jr.:** Funding acquisition, Methodology, Investigation, Writing – review & editing. **Ari Jumpponen:** Funding acquisition, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.121519.

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