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Research Article

RESEARCH ARTICLE

Watershed and fire severity are stronger determinants of soil chemistry and microbiomes than within-watershed woody encroachment in a tallgrass prairie system

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One sentence summary: High-severity burns result in a novel soil microbiome regardless of whether applied to grass-dominated or woody-plant invaded patches in a fire-protected, encroached prairie watershed.

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ABSTRACT

Fire can impact terrestrial ecosystems by changing abiotic and biotic conditions. Short fire intervals maintain grasslands and communities adapted to frequent, low-severity fires. Shrub encroachment that follows longer fire intervals accumulates fuel and can increase fire severity. This patchily distributed biomass creates mosaics of burn severities in the landscape—pyrodiversity. Afforded by a scheduled burn of a watershed protected from fires for 27 years, we investigated effects of woody encroachment and burn severity on soil chemistry and soil-inhabiting bacteria and fungi. We compared soils before and after fire within the fire-protected, shrub-encroached watershed and soils in an adjacent, annually burned and non-encroached watershed. Organic matter and nutrients accumulated in the fire-protected watershed but responded less to woody encroachment within the encroached watershed. Bioavailable nitrogen and phosphorus and fungal and bacterial communities responded to high-severity burn regardless of encroachment. Low-severity fire effects on soil nutrients differed, increased bacterial but decreased fungal diversity and effects of woody encroachment within the encroached watershed were minimal. High-severity burns in the fire-protected watershed led to a novel soil system state distinct from non-encroached and encroached soil systems. We conclude that severe fires may open grassland restoration opportunities to manipulate soil chemistry and microbial communities in shrub-encroached habitats.

Keywords: fire severity and history; woody encroachment; tallgrass prairie ecosystem; soil bacteria and fungi; soil chemistry; alternate ecosystem states

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INTRODUCTION

In terrestrial ecosystems, fire impacts carbon (C), nitrogen (N) and phosphorus (P) cycling (Certini 2005), combusts plant matter before decomposition (Pellegrini *et al.* 2018, 2020) and produces flushes of N and P, making these nutrients more readily available in the short term (Neary *et al.* 1999; Wan, Hui and Luo 2001). Some fire-adapted plants and pyrophilous bacteria and fungi benefit from fire and increase in abundance in the post-fire environment (e.g. Bruns *et al.* 2020; Prendergast-Miller *et al.* 2017; Pulido-Chavez *et al.* 2021). Recovery of microbial populations is crucial to nutrient cycling (Crowther *et al.* 2019) and plant community recovery after fire events (Pressler, Moore and Francesca Cotrufo 2019; Certini *et al.* 2021). Furthermore, variation in characteristics of the fire regime (e.g. fire history and severity) are hypothesized to increase the variability within ecosystems resulting in subsequent increases in diversity within the landscape—the pyrodiversity–biodiversity hypothesis that posits that pyrodiversity begets biodiversity (Martin and Sapsis 1992).

Fire history—some ecosystems are characterized by frequent fires whereas others have decadal or centennial fire intervals (Andela *et al.* 2017). Historically, North American prairies burned every 1–5 years, but fragmentation and fire suppression have resulted in substantial losses of prairie systems (Samson and Knopf 1994; Leach and Givnish 1996) and woody plant encroachment (Ratajczak *et al.* 2014a; Bond 2008; Collins *et al.* 2021). Without frequent fires, shrubs and trees are more likely to thrive by overtopping grasses and expanding root systems to draw in more water and nutrients (Ratajczak *et al.* 2011; Staver, Archibald and Levin 2011; Twidwell *et al.* 2013). The resultant increasingly woody landscape will likely differ in soil communities and below-ground system functions, including carbon sequestration and cycling of other nutrients (Zhou *et al.* 2018). After woody plants establish, the return of fire events may facilitate further encroachment, because post-fire rhizomatous expansion of encroaching woody species allows expedient sprouting to outcompete re-emerging grasses (Ratajczak *et al.* 2011). Woody encroachment can proceed relatively slowly (e.g. 2% increase in woody cover per year; Barger *et al.* 2011) but extended periods without fire can shift vegetation dynamics beyond the point of returning to a grassland state (i.e. hysteresis; Ratajczak, Nippert and Ocheltree 2014b; Bestelmeyer *et al.* 2011; Miller *et al.* 2017; Collins *et al.* 2021) with subsequent impacts on soil chemistry and soil-inhabiting microbial communities.

Fire severity—in grasslands, severe fires can result from an accumulation of woody biomass fuel from shrub encroachment. With less-frequent fires, sporadically distributed downed wood can generate a mosaic of different fire severities in the landscape (Brown *et al.* 2019) resulting in so called ‘pyrodiversity’ (Martin and Sapsis 1992; Jones and Tingley 2021). Fires of different intensities and severities differ in their impacts on biotic and abiotic soil attributes (Certini *et al.* 2021). Compared to low-severity fires, high-severity fires cause greater heat pulses that penetrate deeper into the soil profile (Smith, Cowan and Fitzgerald 2016), impacting soil chemistry (Tomkins *et al.* 1991; Close *et al.* 2011) and soil-inhabiting communities (Reazin *et al.* 2016; Smith *et al.* 2017; Whitman *et al.* 2019; Certini *et al.* 2021). Environmental change and anthropogenic drivers have altered fire frequencies and severities (Andela *et al.* 2017; Pellegrini *et al.* 2018), resulting in a need to better understand the impacts of differing fire severities on soil systems. Critical research gaps exist on how fire severity impacts soil nutrients and soil microbial communities in local and landscape contexts (Certini *et al.* 2021), especially in grassland systems with different histories of fire suppression and woody encroachment.

In this study, we exploited a rare opportunity when a shrub-encroached, grassland watershed that had been protected from fire for 27 years was burned in 2017. This allowed us to compare two, adjacent watersheds with distinct fire histories (i.e. annually burned watershed vs. fire suppressed) for the past 27 years. We also manipulated fuel loads within and outside established patches of woody vegetation within the shrub-encroached watershed to evaluate the effects of woody encroachment as well as high and low-severity fires on soil chemistry as well as on soil bacterial and fungal communities. Our experimental design (Figure S1, Supporting Information) aimed to address effects of fire history, woody encroachment and recent fires of differing severities.

For clarity, we present our study as three distinct research questions (Q1–Q3). First, to explore the potential effects of fire history across the watersheds, we compared grass-dominated experimental units with no woody vegetation (Figure S2A and S2B, Supporting Information) assigned to low-severity fire treatments in two watersheds with distinct fire histories—one burned annually, another with continuous fire suppression for 27 years (Q1). We hypothesized that fire exclusion would shape soil abiotic and biotic attributes and that low-severity fire would have little effect regardless of landscape context. Second, to test local effects of woody encroachment, we compared experimental units within and outside established shrub islands (Figure S2B and S2C, Supporting Information) assigned to low-severity fire treatments within the fire-suppressed watershed (Q2). As in Q1, we hypothesized that the abiotic and biotic soil attributes within established shrub islands would differ from non-encroached grass plots and that low-severity fire would have little effect regardless of vegetation. Third, to test the effect of fire severity and its dependency on the vegetation context, we compared shrub-encroached and grass-dominated experimental units in the fire-protected watershed with two fire severity (low vs. high severity) treatments (Q3; Figure S2B–S2E, Supporting Information). We hypothesized that the high-severity fire would impact abiotic and biotic soil attributes similarly and regardless of vegetation, whereas low-severity fire would have a lesser impact on the abiotic and biotic attributes and depend on local vegetation.

MATERIALS AND METHODS

Study site

The study was conducted at the Konza Prairie Biological Station (KPBS, 39å05’ N, 96å35’ W), which hosts a Long-Term Ecological Research (LTER) site representative of native tallgrass prairie in the Flint Hills of KS, USA. The site spans 3487 ha and remains undisturbed by row-crop agriculture. The vascular flora at KPBS (Towne 2002) is dominated by native C_4 grasses: big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*) and switchgrass (*Panicum virgatum*). The Flint Hills and KPBS are characterized by shallow soils overlaying chert-bearing limestones and shales (Ransom *et al.* 1998). Our study site is classified as typical chernozem according to the Food and Agriculture Organizations (FAO) soils classification used by the United Nations. Topographic relief divides the landscape into upland plateaus with shallow soils, slopes with outcrops of limestone and lowlands with deeper alluvial and colluvial soils. January mean temperature is −3°C (range −9 to 3°C), and the July mean is 27°C (20–33°C). Annual precipitation averages 835 mm, 75% of which falls in the growing season between April and October. Historic fire intervals in the region ranged from 3 to 4 years (Knapp *et al.* 1998), while current and established conservation and rangeland management regimes use annual burning to suppress woody encroachment that threatens the tallgrass prairie ecosystems (Ratajczak *et al.* 2014a; Kollmorgen and Simonett 1965).

Experimental design

More than 20% of the 24 ha fire-protected watershed was covered by woody shrubs in the early 2000s (Briggs *et al.* 2005), but woody cover has since increased and exceeded 50% at the time of burning (Ratajczak *et al.* 2014a). The invading woody shrubs are mainly roughleaf dogwood (*Cornus drummondii*) and staghorn sumac (*Rhus glabra*) with intermittent wild plum (*Prunus americana*) and some other less frequent species (e.g. eastern red cedar—*Juniperus virginiana*; Briggs *et al.* 2005).

Within the fire-protected watershed, we selected a total of 10 established *C. drummondii* shrub islands (ranging from ∼5 to 10 m in diameter) and set up two plots (1 m × 2 m) within each island: one assigned to low-severity fire treatment (treatment 1 in Figure S1, Supporting Information) and the other to high-severity treatment (treatment 2). We also established similar plots outside of established shrub islands that were subjected to low (treatment 3) and high-severity fire treatment (treatment 4). The high-severity fire treatments were created by amending plots with 0.6 m^3 of supplemental small-diameter timber (native *Quercus* spp.), whereas low-severity treatments received no additional fuel. To compare the effects of the woody encroachment to a non-encroached watershed, we selected another, adjacent 35.9 ha watershed that had been annually burned each spring for the past three decades. Annual or semi-annual burning (i.e. usually every 1–5 years) is a common management practice that mimics historic fire intervals (Kollmorgen and Simonett 1965; Knapp *et al.* 1998; Andela *et al.* 2017). In this non-encroached watershed, we randomly selected ten plots for comparison between the two watersheds with distinct fire histories (treatment 5). This experimental design resulted in a total of 50 experimental units: 10 plots assigned to high-severity treatments within the established *C. drummondii* shrub islands; 10 plots assigned to low-severity treatments within the established shrub islands; 10 plots assigned to high-severity treatments outside the shrub islands; 10 plots assigned to low-severity treatments outside the shrub islands and 10 plots in the annually burned watershed assigned to low-severity treatments (Figure S1, Supporting Information). If not ignited by the broadcast burn, the additional fuel in the high-severity treatments was directly ignited. This was usually necessary within the shrub islands because the fire rarely carried through them.

Soil temperature measurements before, during and after burning

We randomly selected one plot from each of the five treatments to measure heat penetration during fire treatments. At each of the selected plots, we dug a 20 cm deep pit to install soil thermocouples. A type K thermocouple (stainless steel sheathed 24 gauge; Omega Engineering, Inc., Stamford, CT) was inserted at 2-, 5-, 10- and 20-cm depths into the wall of the pit in mid-March 2017 in anticipation of the burn treatment as weather conditions would permit. Each thermocouple was attached by a PVC-insulated, type K extension wire to Omega OMPL-TC data loggers, which were placed in waterproof cases containing desiccant packs and buried in playground sand approximately 2 m outside the plot edge. Soil was replaced into the pit after thermocouple installation. Data Loggers were set to record every 5 min starting on March 15, 2017. Both the fire-protected watershed and the annually burned watershed were broadcast burned on April 15, 2017 as part of scheduled KPBS watershed management. Data loggers were collected on April 20, 2017.

Soil sampling

We sampled all 50 plots 4 days before and 28 days after the fire. Our previous fire-response experiments indicated that responses to fire are rapid and fungal communities turn over within a few weeks following a fire event (Reazin *et al.* 2016). After removing litter or residual coals when present, we sampled two 15 cm deep mineral soil cores from each plot with a 6.35 cm diameter slide hammer impact soil corer with 15 cm plastic liners (AMS Inc., American Falls, ID). Samples were placed in plastic bags and transported on ice to the laboratory where they were manually homogenized and subsampled for soil chemistry and nucleic acid analyses. After homogenization, two subsamples were transferred into 50 mL Falcon tubes (Corning Inc., Corning, NY) and stored at −20°C until further processing.

Soil chemistry analyses

When adequate soil homogenate was available, a 50 g subsample was analysed for soil chemistry at the Kansas State University soil testing laboratory (www.agronomy.k-state.edu/services/soiltesting/). One of the two frozen subsamples was thawed and immediately oven dried overnight at 60°C and ground to pass through a 2 mm sieve. This subsample was further divided for analyses of pH, soil organic matter (SOM), total carbon (TotC), total nitrogen (TotN), total phosphorus (TotP), readily available inorganic nitrogen (ammonium [NH_4^+] and nitrate [NO_3^-]), and plant available phosphorus (Mehlich P). A 10 g subsample was used to measure soil pH directly in a 1:1 soil slurry in deionized water. A total of 1 g of dry soil was used to estimate organic matter content through loss on ignition as described in Combs and Nathan (1998). TotC and TotN were measured using a LECO TruSpec CN combustion analyser (LECO, St. Joseph, MI) on a weight % basis from a 0.45 g subsample of prepared soil. TotP was analysed colorimetrically using a modified Kjeldahl digestion and a flow analyser from a 1 g subsample of prepared soil. Inorganic nitrogen (NH_4^+ and NO_3^-) was colorimetrically estimated from a 2 g subsample extracted with 1 M KCl and cadmium reduction for nitrate (Gelderman and Beegle 1998) and run in separate channels in a flow analyser to measure the ions simultaneously. Plant available phosphorus was estimated from a 2 g subsample using Mehlich 3 soil test extractant (Mehlich 1984) as described in Frank *et al.* (2018).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from ∼0.5 g soil subsamples using E.Z.N.A Soil DNA Kit (Omega Bio-Tek, Norcross, GA) following the manufacturer's instructions and stored at −20°C until PCR amplification. The DNA was quantitated using an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and standardized to 2 ng/μL for PCR amplification. For fungi, we chose the Internal Transcribed Spacer 2 (ITS2) region of the ribosomal RNA gene (Schoch *et al.* 2012) for our analyses. We used the fITS7 (Ihrmark, Bo¨deker and Cruz-Martinez 2012) and ITS4 (White *et al.* 1990) primers with unique 12 bp barcodes in each 5'-end in 50 μL PCR reactions. The volumes and final concentrations of reagents were as follows: 10 μL forward and reverse primer (1 μM), 10 μL template DNA (2 ng/μL), 5 μL dNTP (200 μM) and 0.5 μL (1/2 unit) Phusion Green Hot Start II DNA polymerase (ThermoScientific, Pittsburg, PA), 10 μL of Phusion 5X HF Buffer with 7.5 mM $MgCl_2$ and 14.5 μL molecular grade water. The PCR began with an initial denaturing step for 30 s (98°C) and was followed by 35 cycles with 10 s of denaturing (98°C); 30 s of annealing (54°C); 1 min of extension (72°C) and concluding with a 10 min final extension (72°C). For bacteria, we targeted the highly variable V4 region of 16S ribosomal RNA gene with forward primer 515f and reverse primer 806r (Caporaso *et al.* 2012) appended with 12 bp barcodes in each 5'end in 50 μL reactions. The reaction volumes and conditions were identical to those used for fungi except for the 55°C annealing temperature and 30 PCR cycles used to generate bacterial amplicons.

For fungi and bacteria, amplification of PCR contaminants was determined by a negative PCR control in which templates were replaced with ddH_2O. Each sample was PCR-amplified in triplicate and 30 μL of each amplicon was combined into one per experimental unit. The pooled 90 μL amplicons were purified using the Mag-Bind RxnPure Plus Magnetic Bead Cleanup solution (Omega Bio-Tek) following a modified manufacturer protocol with a 1:1 ratio of PCR product to magnetic bead solution and two rinse steps with 80% ethanol. Following cleanup, a total of 200 ng of amplified DNA per experimental unit was pooled. Because the negative controls yielded little measurable DNA, the entire elution volume from the cleanup (40 μL) was included. Illumina adapters and indices were added using four PCR cycles, KAPA Hyper Prep Kit (Roche, Pleasenton, CA) and 0.5 μg starting DNA. The library was sequenced (2 × 300 cycles) using the Illumina MiSeq Personal Sequencing System at the Integrated Genomics Facility (Kansas State University, Manhattan, KS). The sequence data are available through the Sequence Read Archive under BioProject PRJNA718337, samples SAMN18529080–SAMN18529179.

Sequence data processing

Sequence data were processed using the mothur pipeline (v. 1.38.0; Schloss *et al.* 2009) following the MiSeq standard operating protocol to generate OTUs (Kozich *et al.* 2013). Paired fastq files were contiged, quality-filtered and primer sequences removed. Sequences with > 1 bp difference with the primers or without an exact match to the sample-specific identifiers were omitted. Bacterial and fungal sequences were analysed independently. Bacterial sequences were aligned against a mothur-formatted 16S Silva Alignment (v. 132; www.arb-silva.de), whereas fungal sequences were truncated to the length equal to the shortest high-quality read (237 bp excluding primers and sample-specific identifiers). Bacterial and fungal data were then pseudo single-linkage clustered (99%; Huse *et al.* 2010) to control for platform generated errors and screened for potential chimeras (UCHIME; Edgar *et al.* 2011), with putative chimeras culled. The sequence data were assigned to taxon affinities using mothur-embedded Nai¨ve Bayesian Classifier (Wang *et al.* 2007) using the default cutoff of 80% sequence similarity. Bacterial sequences were screened against the Ribosomal Database Project's 16S reference training set (v.9) and sequences assigned to Eukarya, mitochondria, plastids or unknown were removed. Fungal data were assigned to taxa using the UNITE taxonomy reference (Kõljalg *et al.* 2013) and sequences with no match in the UNITE reference or assigned to Protista and Plantae were removed. A sequence distance matrix was generated for aligned bacterial sequences. Fungal sequences were clustered using vsearch (Rognes *et al.* 2016). Both bacterial and fungal data were assigned to Operational Taxonomic Units (OTUs) at 97% similarity or greater. Rare OTUs represented by fewer than ten sequences in the entire dataset were removed as potential artifacts (Brown *et al.* 2015; Oliver *et al.* 2015).

We estimated Good's coverage (i.e. complement of the ratio between local singleton OTUs and the total sequence count) for each experimental unit to evaluate the representativeness of our sampling. To estimate richness and diversity, we iteratively (100 iterations) calculated observed (S_{Obs}) richness, diversity (i.e. Shannon–Weiner diversity [H']) and evenness (i.e. Shannon's equitability) with subsampled data (1000 fungal and 5000 bacterial sequences per experimental unit), as recommended by Gihring, Green and Schadt (2012) to avoid biased comparisons of diversity and richness estimators in samples with unequal sequence yields. Although five times more bacterial (5000 sequences per sample) than fungal (1000 sequences per sample) sequence data were subsampled, coverage was lower for bacteria (0.85 ± 0.03; mean ± standard deviations) than fungi (0.95 ± 0.02). Fungal coverage estimates were generally high indicating reasonable sampling of soil communities regardless of shallow subsampling.

Statistical analyses

Since the same experimental units were sampled before and after fire, we used repeated measures analyses of variance (ANOVA; Tables S1–S3, Supporting Information). Tukey's post-hoc comparisons were performed for the univariate response variables for each research question. To test the watershed and low-severity fire effects (Q1), we compared only non-encroached plots assigned to low-severity fire treatments before and after the fire in two watersheds with distinct fire histories—one burned annually, another protected from fire for 27 years (treatments 3 and 5 in Figure S1, Supporting Information). These analyses included main effects watershed and time (i.e. before and after fire) and their interaction. To test the effect of woody encroachment (Q2), we compared low-severity fire treatment plots within and outside shrub islands in the fire protected watershed before and after the fire (treatments 1 and 3). These analyses included main effects vegetation and time and their interaction. To test the effect of fire severity and its dependency on the vegetation context (Q3), we compared plots in a fire-protected watershed representing two vegetation types (shrub-encroached vs. grass-dominated) and two fire severity (low vs. high [treatments 2 and 4]) treatment combinations. These analyses included main effects vegetation, severity and time as well as their two- and three-way interactions.

The soil chemistry data, as well as both bacterial and fungal richness and diversity data were non-normal (Shapiro–Wilk Goodness of Fit tests: $W > 0.59$; $P < 0.02$) and heteroscedastic (Welch's tests: $F_{7,34} > 2.79$; $P < 0.04$). As such, these data were in-transformed, except for the proportional community data and % data (OM, total N and total C) that were arcsine-square root transformed.

To visualize and infer compositional differences within bacterial and fungal communities, we calculated pairwise Bray–Curtis distances and visualized these data with Principal Coordinates Analysis (PCoA) using R package 'vegan' (Oksanen *et al.*

2019). The optimal number of dimensions (k) was determined based on stabilizing stress less than 0.20 using 1000 runs with empirical data and a random seed starting value. Community data were compared using a nonparametric permutational analog of traditional analysis of variance (PERMANOVA) (Oksanen *et al.* 2019) using function 'adonis', the 'vegan' implementation of PERMANOVA (Anderson 2001). To address questions about shifts in the degree of variability among groups, we used the function 'betadisper' as the multivariate analog of Levene's test for homogeneity of molecular variances (HOMOVA). Similarities between samples were calculated for PERMANOVA using the function 'betadiver()' method 'beta.z'. Function 'envfit' was used to explore goodness of fit (r^2) correlations of soil chemistry variables with PCoA axes created for pairwise distances among soil microbiome samples (Table S4, Supporting Information). To identify OTUs associated with observed community differences, if any, we performed indicator species analysis with package 'indicspecies' (Cáceres and Legendre 2009) using function 'multipatt' (alpha = 0.05) to test associations between taxa and treatments for each of our three research questions for the 100 most abundant bacterial and fungal OTUs. To visualize trends in taxonomic level indicators of change, heatmaps were created using package 'ampvis2' (Andersen *et al.* 2018). Heatmaps included the ten most abundant bacterial and fungal OTUs by % read abundance within samples corresponding to each research question.

RESULTS

The fuel addition resulted in a distinct peak in soil temperatures. The maximum temperatures in the high-severity fire treatment at 2 cm measured 94.9°C outside the established shrubs and 78.6°C within. The heat pulse was ephemeral, as > 60°C temperatures were maintained for 10 or 11 h at a depth of 2 cm in the two plots with instrumentation. Heat penetration from the high-severity fires declined rapidly with soil depth. Although high maximum temperatures were measured in both instrumented plots at 5 cm depth (84.7°C outside and 68.4°C within the shrub island), the maximum at 10 cm was only 39°C outside the shrub island, whereas the 10 cm thermocouple within the shrub was lost during the experiment. At 20 cm, the temperature differences in the high and low-severity treatments were relatively small: 24.0°C in high and 20.8°C in low-severity treatment outside a shrub island, or 25.6°C in high and 17.8°C in low-severity treatment within a shrub island. The low-severity fire treatment did not generate a strong heat pulse, although the maximum temperatures during the fire were higher at 2, 5 and 10 cm than they were either before or after the fire. Notably, a warm day on April 2, roughly 2 weeks prior to the fires, resulted in higher peak temperatures in the topmost soil profiles than those observed during the fire in low-severity treatments in the non-encroached watershed. There was no visible heat pulse in the low-severity treatment within the shrub island. However, the low-severity fire often did not carry through the established shrub island.

Q1: Responses to fire frequency and low-severity fire across watersheds

Soil chemistry was distinct between the fire-protected and annually burned watersheds but largely unaffected by the low-severity fire (Fig. 1A–H; Table S1, Supporting Information). TotC ($F_{1,18} = 12.9$, $P = 0.002$), TotN ($F_{1,18} = 19.0$, $P < 0.001$), inorganic N (NH_4^+—$F_{1,18} = 22.5$, $P < 0.001$; NO_3^-—$F_{1,18} = 8.54$, $P = 0.009$) and TotP ($F_{1,18} = 7.59$, $P = 0.013$) were higher in the fire-protected watershed than in the annually burned watershed. Soil NH_4^+ was higher in the fire-protected watershed than in the annually burned watershed but decreased after low-severity fire ($F_{1,18} = 22.8$, $P < 0.001$; Fig. 1F). In contrast to NH_4^+, Mehlich P increased after fire ($F_{1,18} = 75.5$, $P < 0.001$) but was not observed to differ between watersheds. In addition, we observed neither a change in soil pH after low-severity fire ($F_{1,18} = 2.23$, $P = 0.143$) nor a soil pH difference between watersheds ($F_{1,18} = 0.06$, $P = 0.816$).

Soil bacterial and fungal richness and diversity responded differently across watersheds and low-severity fire. Bacterial richness ($F_{1,18} = 15.1$, $P = 0.001$) and diversity ($F_{1,18} = 16.8$, $P < 0.001$) increased after the low-severity fire (Fig. 2A and B; Table S1, Supporting Information) with neither any evidence for differences in bacterial richness ($F_{1,18} = 0.10$, $P = 0.754$) and diversity ($F_{1,18} = 0.03$, $P = 0.857$) between watersheds nor for interactions between watershed and low-severity burn (Table S1, Supporting Information). Fungal richness ($F_{1,16} = 45.4$, $P < 0.001$) and diversity ($F_{1,16} = 41.4$, $P < 0.001$) were both greater in the encroached watershed compared to the annually burned grassland (Fig. 2C and D; Table S1, Supporting Information). Fungal richness was not affected by the low-severity fire ($F_{1,16} = 2.88$, $P = 0.109$; Fig. 2D), and similar to bacterial richness and diversity, we observed no evidence of an interaction between watersheds and the low-severity fire (Table S1, Supporting Information).

Both bacterial (PERMANOVA: $F_{1,39} = 3.40$, $P < 0.001$) and fungal ($F_{1,37} = 6.70$, $P < 0.001$) communities differed compositionally between the annually burned and fire-protected watersheds (Fig. 3A and B). Watershed explained 15% of the variation (r^2) in fungal turnover and 8% for bacteria. Fungal communities (PERMANOVA: $F_{1,37} = 1.98$, $P < 0.01$) also differed before and after fire, whereas bacterial communities did not (PERMANOVA: $F_{1,39} = 1.98$, $P = 0.063$). The low-severity fire accounted for 5% of variation between pre- and post-fire fungal communities. We observed no evidence for shifts in the degree of variability among bacterial (HOMOVA: $F_{3,36} = 0.44$, $P = 0.724$) or fungal (HOMOVA: $F_{3,34} = 1.72$, $P = 0.182$) communities after the low-severity fire or between watersheds (Fig. 3A and B). Bacterial communities correlated more strongly with soil pH and TotP relative to other soil chemistry variables fitted onto community pairwise distances in ordination space (Figure S3A, S3B and Table S4, Supporting Information). Conversely, fungal but not bacterial, communities correlated strongly with NH_4^+—the only high correlate of community change that also differed by watershed and fire treatment (Fig. 1F).

Among the 100 most abundant bacterial and fungal OTUs, indicator taxon analyses identified 17 bacterial and 50 fungal indicators that were significantly ($P < 0.05$) more frequent in one of the four watershed-by-time treatment combinations (Table S5, Supporting Information). Proteobacteria were most abundant among the indicator taxa (7 of 17 watershed-by-time indicator OTUs), whereas no bacterial families were clearly disproportionately more abundant in any treatment combination (Figure S4, Supporting Information). Ascomycota dominated among fungal watershed indicator OTUs (36 of 50), especially in the fire-protected watershed before fire. *Neurospora terricola* (Ascomycota) responded to fire positively within the fire-protected watershed, but only minimally in the annually burned watershed (Figure S4, Supporting Information). *Malassezia* (Basidiomycota) increased 6-fold in the annually burned watershed post-fire compared to a 2-fold increase in the encroached watershed. The family Mortierellaceae (Mucoromycota) was represented by 3 of the 10 most abundant OTUs in these analyses, including *Mortierella exigua*, which was more abundant in the fire-protected watershed but did not change in abundance after

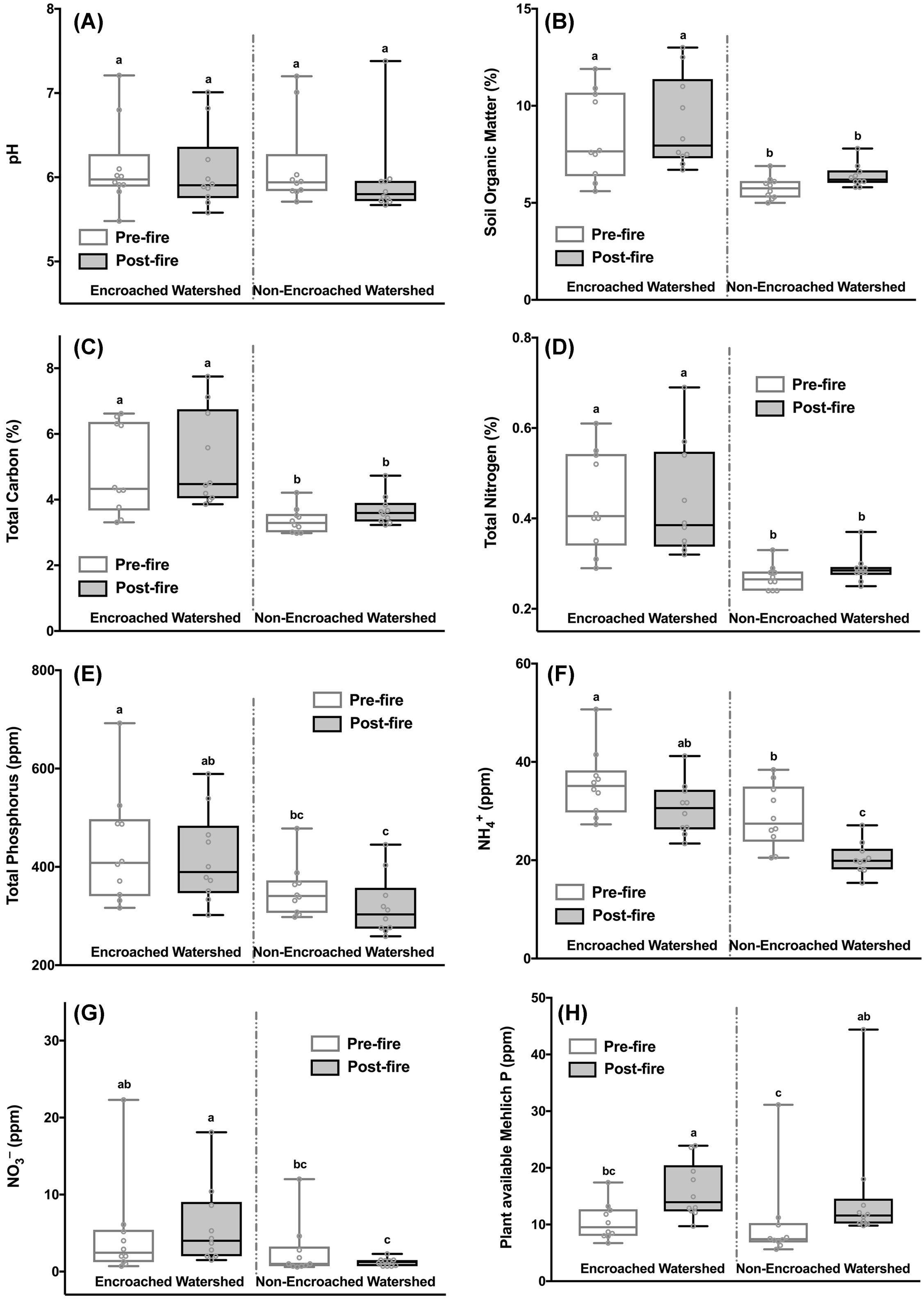

Figure 1. (A)–(H). Soil (A) pH, (B) organic matter, (C) TotC, (D) TotN, (E) TotP, (F) ammonium, (G) nitrate and (H) plant available (Mehlich) phosphorus in watersheds that had been either protected from fire and encroached by *C. drummondii* shrubs (encroached) or burned annually and void of *C. drummondii* encroachment (non-encroached) 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q1 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes watershed (W) and time (T) main effects as well as their interaction (W × T).

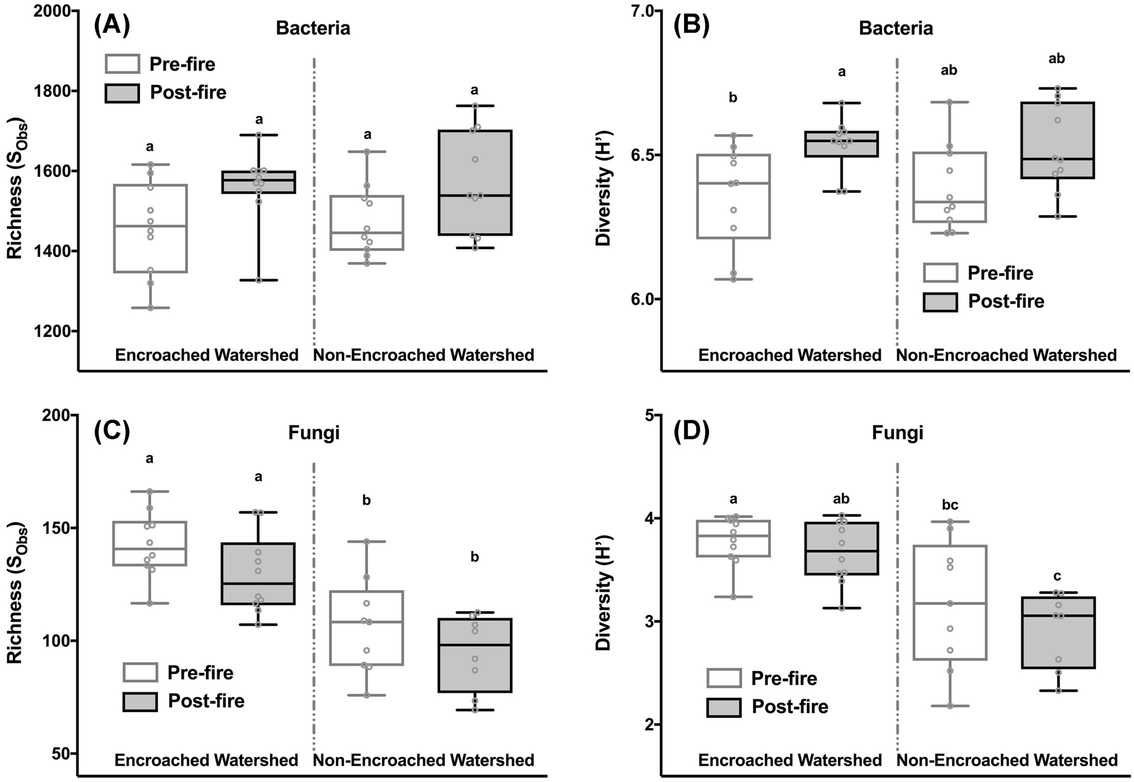

Figure 2. (A)–(D). Bacterial (A) and (B) and fungal (C) and (D) observed (S_{Obs}—A and C) richness and diversity (Shannon's *H'*—B anbd D) in watersheds that had been either protected from fire and encroached by *C. drummondii* shrubs (encroached) or burned annually and void of *C. drummondii* encroachment (non-encroached) 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q1 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes watershed (W) and time (T) main effects as well as their interaction (W × T).

fire. In contrast, *Mortierella rishikesha* was more abundant in the annually burned watershed and decreased after the fire (Figure S4, Supporting Information). Analyses focusing on pre- vs. post-fire indicators (i.e. pooled across watersheds) revealed only one bacterial indicator and no fungal indicators for post-fire samples (Table S5, Supporting Information). Comparing watersheds (i.e. pooled across pre- and post-fire samples), however, we identified 45 fungal and 17 bacterial indicator OTUs that were disproportionately more abundant in the fire-protected watershed, compared to just four fungal and two bacterial indicators in the annually burned watershed (Table S5, Supporting Information).

Q2: Responses to woody encroachment and low-severity fire within a fire-protected watershed

Most measured soil variables did not differ between shrub-encroached and grass-dominated patches within the fire-protected watershed and seemed unresponsive to low-severity fire (Fig. 4A–H; Table S2, Supporting Information). The exception was NH_4^+, which declined after the fire ($F_{1,18} = 6.01$, $P = 0.025$) but did not differ between *C. drummondii* islands and grass-dominated patches ($F_{1,18} = 2.91$, $P = 0.105$), with no evidence of a significant interaction ($F_{1,18} = 0.22$, $P = 0.643$). Plant available Mehlich P increased after low-severity fire ($F_{1,18} = 45.9$, $P < 0.001$) but was unaffected by vegetation ($F_{1,18} = 0.29$, $P = 0.597$; Fig. 4H), as was the case between watersheds (Q1).

Within the fire-protected watershed, bacterial and fungal richness and diversity responded to low-severity fire but was not affected by local vegetation (Fig. 5A–D; Table S2, Supporting Information). Bacterial richness ($F_{1,18} = 15.7$, $P < 0.001$) and diversity ($F_{1,18} = 14.7$, $P = 0.001$) were higher after the low-severity fire (Fig. 5A and B). We observed a decline in fungal richness following the fire ($F_{1,18} = 15.8$, $P < 0.001$), but there was no evidence for change in fungal diversity ($F_{1,18} = 0.67$, $P = 0.424$; Fig 5C and D).

There was no strong evidence that low-severity fire (PERMANOVA: $F_{1,39} = 1.57$, $P = 0.058$) or vegetation ($F_{1,39} = 1.37$, $P = 0.108$) affected bacterial community composition (Fig. 3C). In contrast, fungal communities differed within and outside established shrub islands (PERMANOVA: $F_{1,39} = 2.27$, $P < 0.001$) as well as before and after fire ($F_{1,39} = 1.80$, $P < 0.001$; Fig. 3D). Vegetation and fire explained 6% and 4% of fungal community variation, respectively. As in Q1 comparing encroached and non-encroached watersheds, we observed no interaction between local shrub encroachment and low-severity fire for either bacterial (PERMANOVA: $F_{1,39} = 0.635$, $P = 0.983$) or fungal (PERMANOVA: $F_{1,39} = 0.88$, $P = 0.773$) community responses. In addition, there was no evidence for shifts in the degree of variability among bacterial (HOMOVA: $F_{3,36} = 0.86$, $P = 0.470$) or fungal (HOMOVA: $F_{3,36} = 1.65$, $P = 0.194$) communities among vegetation or pre- and post-fire samples (Fig. 3C and D). Both bacterial and fungal communities correlated with soil pH at the local scale (Figure S3C, S3D and Table S4, Supporting Information), which was not the case for fungal communities across watersheds (Q1). Except for pH, soil chemistry correlation coefficients were higher for fungal than bacterial communities. Among the high correlates, only NH_4^+ (Fig. 4F) and Mehlich P (Fig. 4H) were affected by fire treatment.

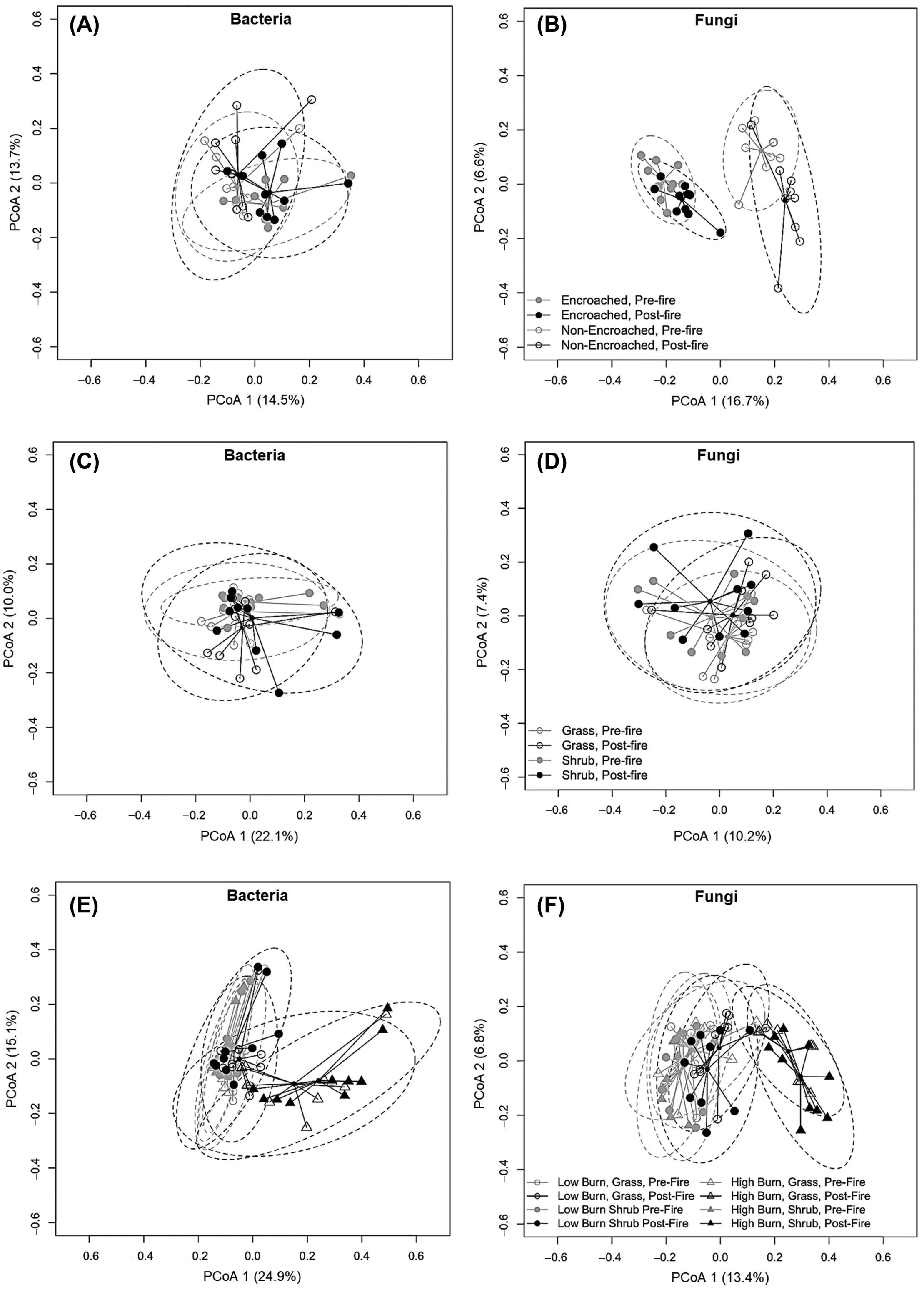

Figure 3. (A)–(F). Bacterial and fungal mean community compositions (shapes) and dispersions (solid lines) with 95% confidence intervals (broken-line clouds) for comparing samples in: Q1 (A) and (B) from the encroached and non-encroached watershed before and after burning; Q2 (C) and (D) from shrub islands and grass plots before and after burning in the non-encroached watershed; Q3 (E) and (F) from shrub islands and grass plots in the non-encroached watershed before and after either high or low-severity burning. Community divergence or convergence measured as median distances to centroid with groups compared using ANOVA.

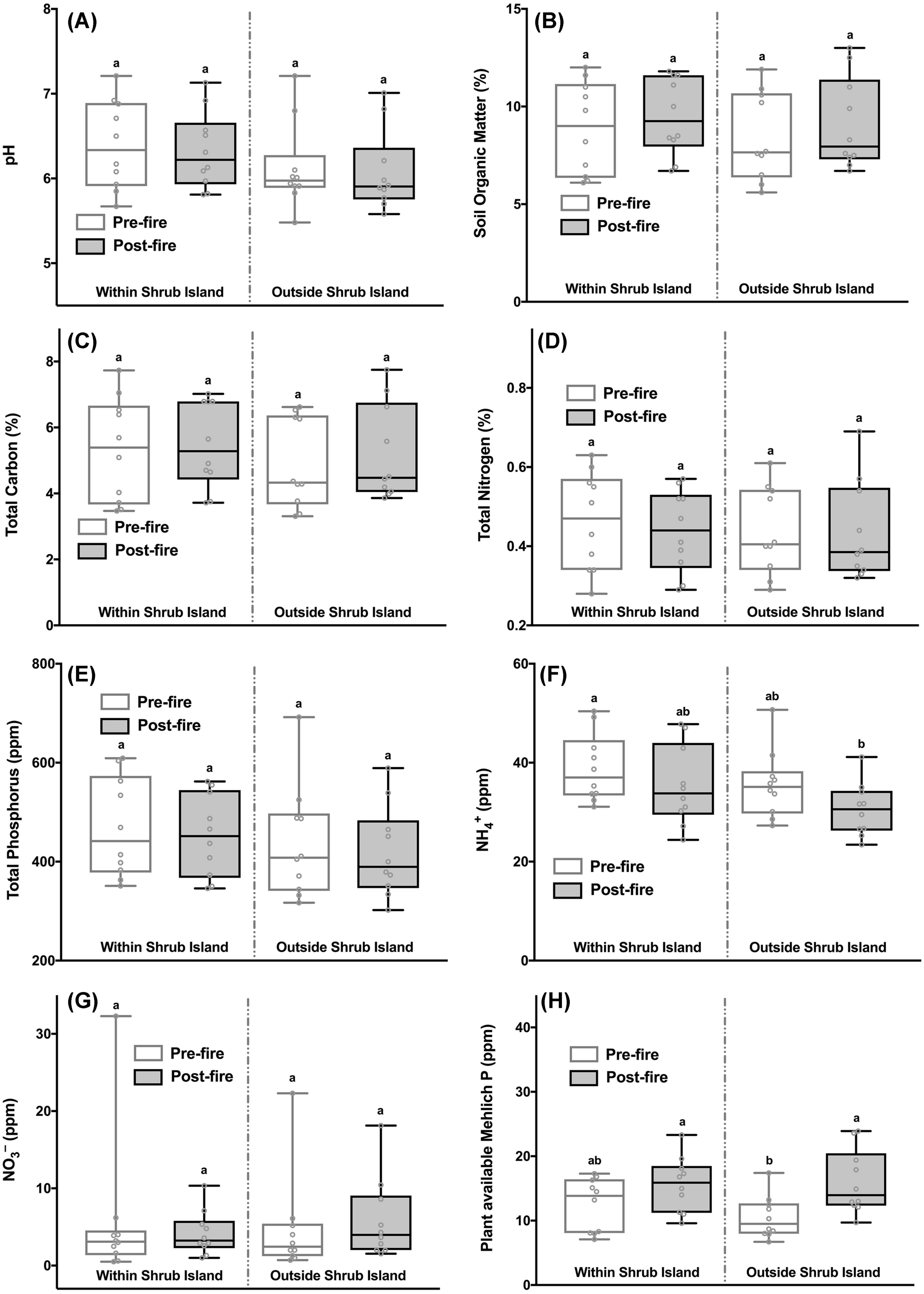

Figure 4. (A)–(H). Soil (A) pH, (B) organic matter, (C) TotC, (D) TotN, (E) TotP, (F) ammonium, (G) nitrate and (H) plant available (Mehlich) phosphorus in watershed encroached by *C. drummondii* shrubs within shrub islands or outside shrub islands 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q2 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes location relative to shrub island (S) and time (T) main effects as well as their interaction (S × T).

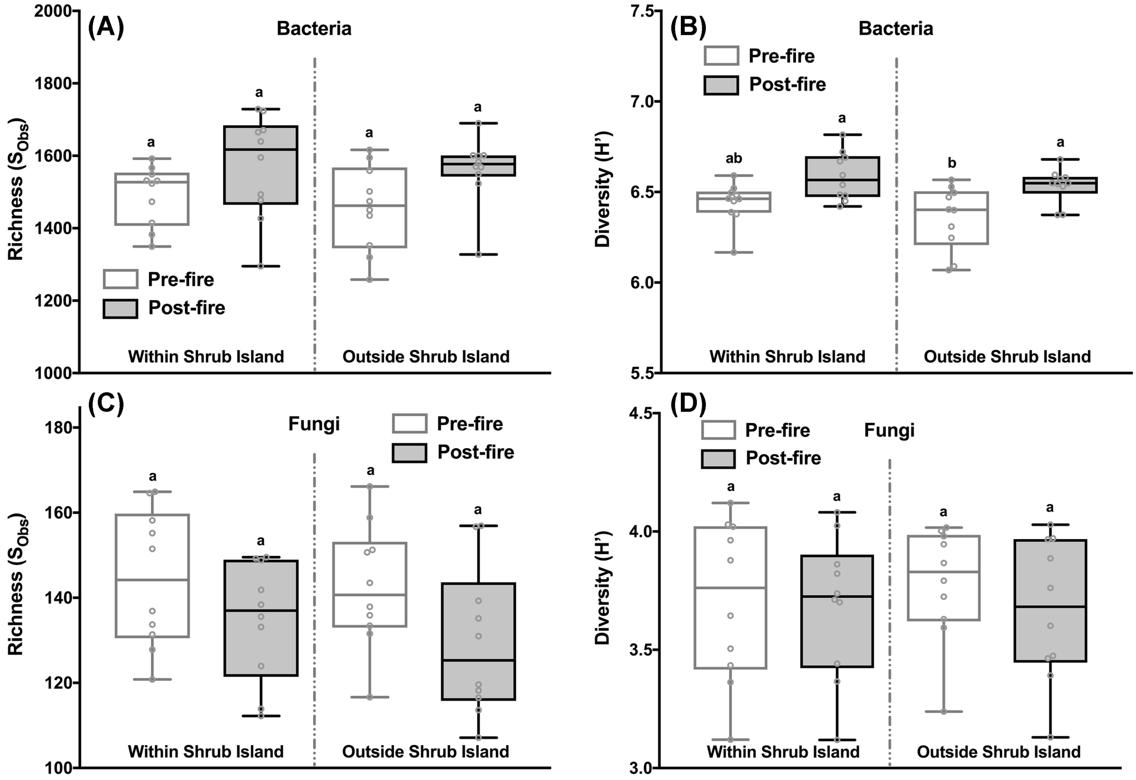

Figure 5. (A)–(D). Bacterial (A) and (B) and fungal (C) and (D) observed (S_{Obs}—A and C) richness and diversity (Shannon's *H'*—B and D) in watershed encroached by *C. drummondii* shrubs within shrub islands or outside shrub islands 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q2 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes location relative to shrub island (S) and time (T) main effects as well as their interaction (S × T).

We identified 30 bacterial and 14 fungal indicator OTUs among the four vegetation-by-time combinations (Table S6, Supporting Information). Like Q1, when assessing the pre- or post-fire indicators, there were few indicators of the low-severity fire—two bacterial and no fungal OTUs. When assessing bacterial indicators of vegetation, there was little change overall at the phylum (Table S6, Supporting Information) or family level (Figure S5, Supporting Information). Fungal OTUs that were abundant in the Q1 indicator analyses were also abundant for Q2, and responses to fire among the most abundant OTUs were similar but less pronounced (Figure S5, Supporting Information). For example, *Malassezia* sp. (OTU3) doubled in read abundance after low-severity fire in the encroached watershed grass habitat but increased 6-fold after fire within the annually burned, non-encroached watershed (Figure S4, Supporting Information).

Q3: Responses to woody encroachment and high-severity fire

Fire severity and vegetation rarely revealed interactions with time among soil chemistry responses(Fig. 6A–H; Table S3, Supporting Information). However, soil pH and NH_4^+ changed differently after low and high-severity burn treatments. Soil pH was marginally higher within shrub islands ($F_{1,36} = 3.52$, $P = 0.069$), after fire ($F_{1,36} = 37.1$, $P < 0.001$) and after the high-severity fire ($F_{1,36} = 51.4$, $P < 0.001$). Dissection of the within subject interaction terms indicated that the post-fire soil pH increase was mostly attributable to the high-severity fire treatment (Fig. 6A). Like pH, NH_4^+ concentration was higher within the shrub islands ($F_{1,36} = 6.02$, $P = 0.019$), after fire ($F_{1,36} = 24.3$, $P < 0.001$) and after high-severity fire ($F_{1,36} = 47.2$, $P < 0.001$), as there were no comparable responses to low-severity fire (Fig. 6F). Mehlich P did not vary with vegetation ($F_{1,36} = 1.06$, $P = 0.311$), but was higher after fire ($F_{1,36} = 207.8$, $P < 0.001$), and after high-severity fire ($F_{1,36} = 82.7$, $P < 0.001$). The significant within subject interaction between time and burn severity was attributable to the greater increase in Mehlich P after the high-severity burn than after the low-severity one (Fig. 6H).

Richness and diversity analyses indicated community fire responses similar to soil chemistry: pre- and post-fire responses depended on fire severity but not vegetation (Fig. 7A–D; Table S3, Supporting Information). Bacterial ($F_{1,36} = 41.0$, $P < 0.001$) and fungal richness ($F_{1,35} = 70.8$, $P < 0.001$), and diversity (bacteria—$F_{1,36} = 43.2$, $P < 0.001$; fungi—$F_{1,35} = 77.4$, $P < 0.001$) declined substantially after high-severity fire. Fungal richness was more sensitive to the severe fire and declined by ~60% (Fig. 7C), whereas bacterial richness declined by ~23% (Fig. 7A). Like Q2, there was no evidence that either bacterial or fungal richness and diversity were affected by shrub encroachment.

Bacterial (PERMANOVA: $F_{1,78} = 2.79$, $P < 0.001$) and fungal ($F_{1,77} = 5.91$, $P < 0.001$) communities also responded compositionally to high-severity fire treatments. A total of 6% of bacterial and 3% of fungal community composition was explained by the interaction between fire severity and time (Fig. 3E and F). In addition, we found evidence for shifts in the degree of variability for both bacterial and fungal communities after the high-severity fire as measured by median distances from ordination centroids (Fig. 3E and F). Bacterial communities were more heterogenous in their composition after high-severity fires both outside ($P_{adj.}$

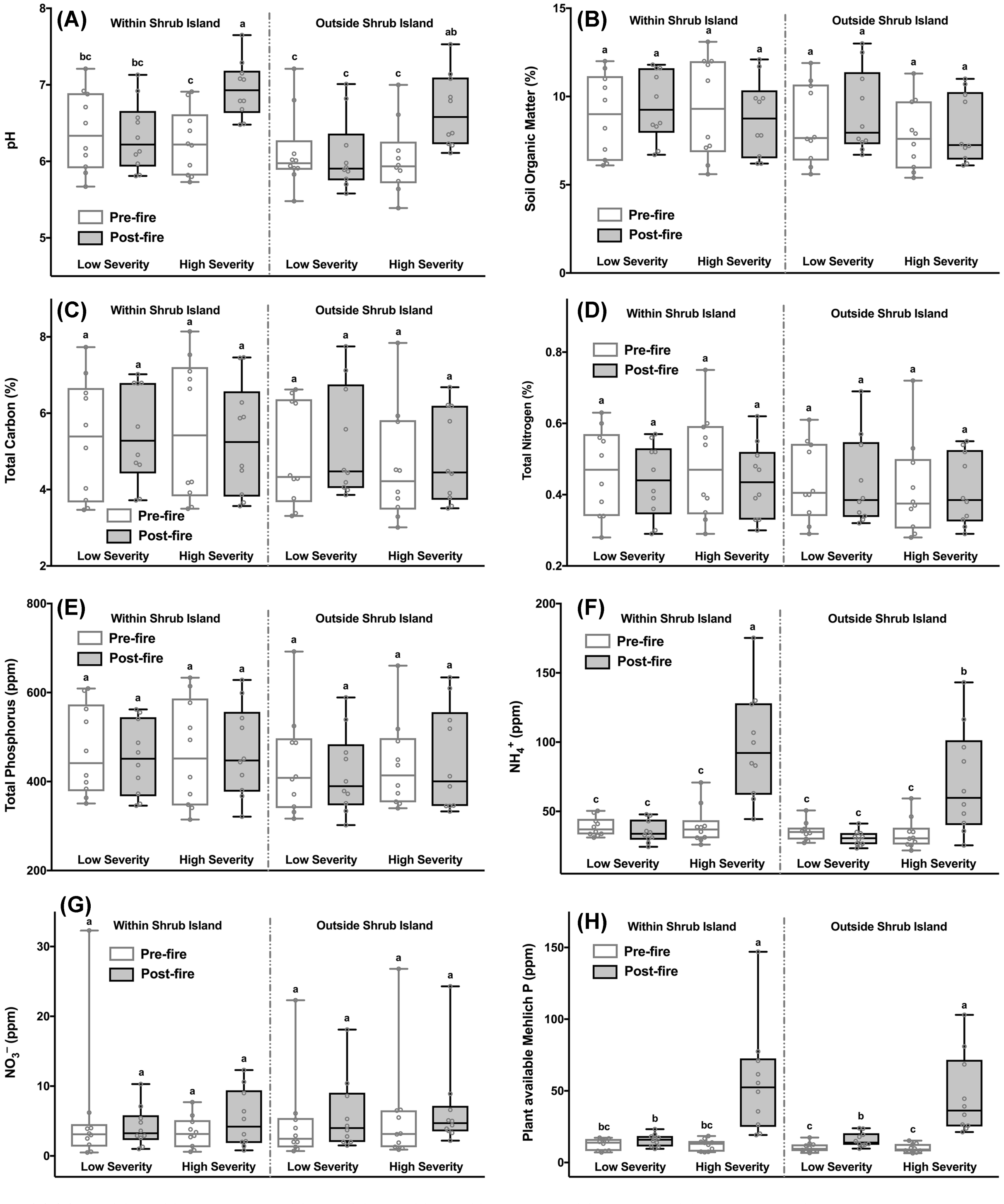

Figure 6. (A)–(H). Soil (A) pH, (B) organic matter, (C) TotC, (D) TotN, (E) TotP, (F) ammonium, (G) nitrate and (H) plant available (Mehlich) phosphorus in watershed encroached by *C. drummondii* shrubs within shrub islands or outside shrub islands burned by low or high-severity fire 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q3 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes location relative to shrub island (V), fire severity (S) and time (T) main effects as well as two- and three-way interactions.

= 0.017) and within shrub islands ($P_{adj.} < 0.001$) based on pairwise Tukey's post-hoc comparisons. Fungal communities were compositionally more heterogenous within shrub islands ($P_{adj.} = 0.036$) and marginally so outside them ($P_{adj.} = 0.055$) after the high-severity fire (Fig. 3E and F). Mehlich P and NH_4^+ correlated with both bacterial and fungal community shifts after high-severity fire (Figure S3E, S3F and Table S4, Supporting Information), whereas NO_3^- correlated with neither. These three soil chemistry correlates were also affected by the severity-by-time interaction (Fig. 6).

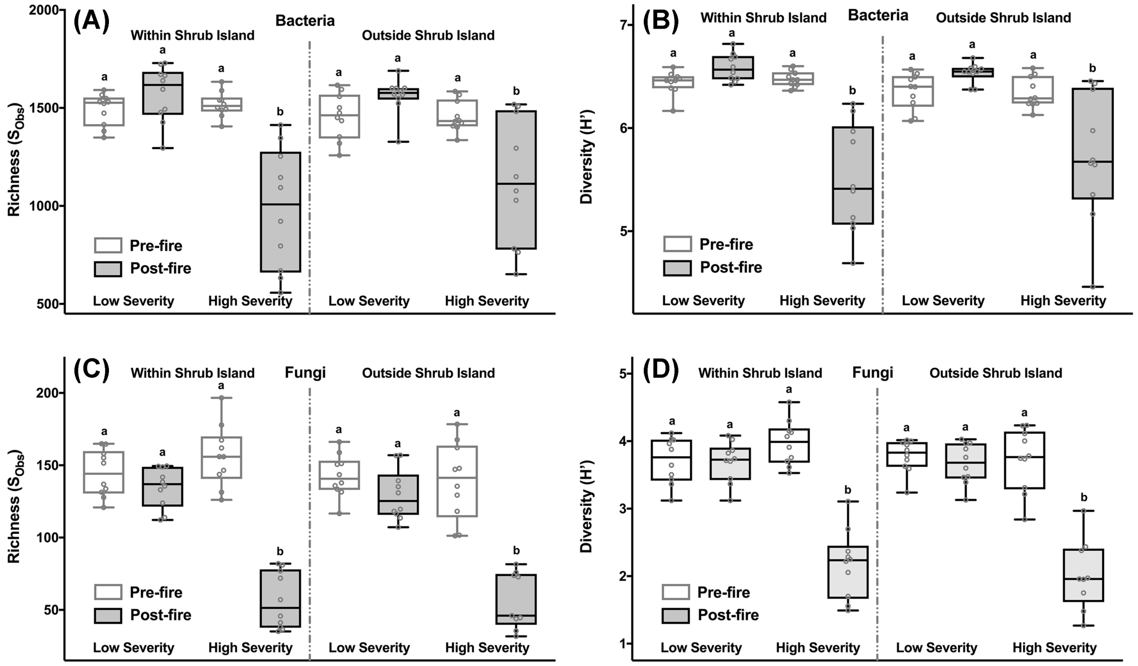

Figure 7. (A)–(D). Bacterial (A) and (B) and fungal (C) and (D) observed (S_{Obs}—A and C) richness and diversity (Shannon's *H'*—B and D) in watershed encroached by *C. drummondii* shrubs within shrub islands or outside shrub islands burned by low or high-severity fire 4 days before (pre-fire) and 28 days after (post-fire) prescribed burning (Q3 in text). Letters show the Tukey post-hoc results of Analysis of Variance for a model that includes location relative to shrub island (V), fire severity (S) and time (T) main effects as well as two- and three-way interactions.

To further investigate how bacterial and fungal community compositions were affected by the severity-by-time interaction, we analysed: pre-fire communities in low and high-severity treatments; post-fire communities in low and high-severity treatments; low fire severity communities before and after fire and high fire severity communities before and after fire. Before the fire, communities in low and high-severity treatments were indistinguishable (PERMANOVA: bacteria—$F_{1,38} = 0.95$, $P = 0.46$; fungi—$F_{1,38} = 0.73$, $P = 0.99$). After fire, these communities were distinct or marginally so: low-severity fire affected the bacterial (PERMANOVA: $F_{1,38} = 1.57$, $P = 0.06$) and fungal communities ($F_{1,37} = 1.74$, $P = 0.004$). Evidence for community dispersion after low-severity fire was marginal as well (bacteria—$F_{1,38} = 2.12$, $P = 0.15$; fungi $F_{1,38} = 4.75$, $P = 0.04$). In contrast, the high-severity fires drove divergence in both bacterial ($F_{1,38} = 37.4$, $P < 0.001$) and fungal ($F_{1,37} = 12.7$; $P < 0.001$) communities and changed their overall composition (PERMANOVA: bacteria—$F_{1,38} = 12.6$; $P < 0.001$; fungi—$F_{1,37} = 7.22$; $P < 0.001$).

We also investigated microbial communities across local vegetation in the context of fire severity. Within and outside the established shrubs, the pre-fire bacterial communities did not differ compositionally before low (PERMANOVA: $F_{1,18} = 1.07$, $P = 0.32$) or high ($F_{1,18} = 1.22$, $P = 0.21$) severity fire treatments. After fire, regardless of severity, bacterial communities remained indistinguishable within and outside shrub islands (PERMANOVA: low severity—$F_{1,18} = 0.95$, $P = 0.46$; high severity—$F_{1,18} = 0.97$, $P = 0.41$), but were distinct from those before the fire (see above). In contrast, fungal communities were distinct within and outside shrub islands before fire (PERMANOVA: low severity—$F_{1,18} = 1.66$, $P = 0.003$; high severity—$F_{1,18} = 1.65$, $P = 0.002$), as well as after the low-severity fire ($F_{1,18} = 1.50$, $P = 0.001$). However, fungal communities were no longer distinct within and outside shrub islands after the high-severity fire ($F_{1,17} = 1.09$, $P = 0.21$) such that the high-severity fire homogenized the fungal community composition across the vegetation patches. For both bacteria and fungi, there were no significant differences in community dispersion within or beyond shrub islands before or after the two experimental fire severities.

Across the eight treatment combinations addressing Q3, we identified three indicators—all three were fungi associated with high-severity burning (Table S10, Supporting Information). One fungal indicator (OTU10, *Pyronema* sp.) was disproportionately more abundant after high-severity fire in shrub islands. In a BLAST search, the *Pyronema* sp. OTU10 matched closely with pyrophilous *Pyronema omphalodes* (GenBank accession MK886722.1) and *Pyronema domesticum* (GenBank accession MT816457.1) with equal 100% sequence similarity and 100% query coverage. The other two indicators (OTU7, *Scutellinia* sp.; OTU44, *Penicillium melinii*) were disproportionately more abundant after high-severity fire regardless of vegetation. The *Scutellinia* OTU7 matched closely *Anthracobia* sp. in Pyronemataceae (100% query coverage and 100% sequence similarity to GenBank accession MK840934.1)—a likely pyrophilous fungus (Claridge, Trappe and Hansen 2009; Hughes *et al.* 2020). To focus on fire effects, we also analysed samples from within and outside the shrub islands together. We identified the same three fungal indicators (described above), as well as two high-severity, post-fire bacterial indicators (OTU54 and OTU79) within the family Moraxellaceae (Table S9, Supporting Information). Among the 10 most abundant bacteria in these analyses, OTU7 (Micromonosporaceae) and OTU6 (Planococcaceae) responded positively to high-severity fire and increased 20- and 60-fold in read abundance, respectively. In contrast, OTU5 (Bradyrhizobiaceae) and OTU8 (Gaiellaceae) decreased by 65% and 54% after high-severity fire, respectively (Figure S6, Supporting Information). Fungal responses to low and high-severity fires also differed. For example, *N. terricola* (OTU1) and *Alternaria porri* (OTU5) increased in abundance twenty and 6-fold, respectively, after high-severity fire, whereas both only increased 3-fold after low-severity fire (Figure S6, Supporting Information). In addition, *M. rishikesha* (OTU2) and *M. exigua* (OTU4) both decreased more dramatically after high-severity than low-severity fire.

DISCUSSION

Adjacent watersheds with different fire histories had distinct soil chemistries and microbial communities that were rarely altered after a low-severity burn. We acknowledge that our experimental design does not account for the potential spatially confounding factors that may have attributed to the observed watershed-level differences. Notwithstanding, we emphasize that indeed, it is often fire history and management that drives ecosystem dynamics in this tallgrass prairie system, rather than typical low-severity prescribed fire events. Within the fire-protected watershed, high-severity fires led to dramatic changes in soil chemistry as well as in bacterial and fungal communities. These changes were minimal or absent after low-severity fires and were rarely affected by woody encroachment within the fire-protected watershed.

In our study, only high-severity fire affected soil chemistry and microbial communities similarly regardless of woody encroachment suggesting that high-severity fire can homogenize abiotic and biotic grassland attributes. Bacterial and fungal diversity and community composition often correlated with changes in the soil chemistry, although this was not true for NO_3^- after high-severity fires. In addition, soil pH correlated with observed differences in bacterial communities but not fungal communities between the annually burned and fire-protected watersheds. Instead, changes in SOM correlated with changes in fungal communities, and soil pH correlated with bacterial communities after both low- and high-severity fires. Soil chemistry is a critical determinant of soil-inhabiting communities post-fire (Certini *et al.* 2021), and bacteria and fungi mediate major biogeochemical transformations including decomposition, soil fertility and C storage (Crowther *et al.* 2019). Understanding the effects of fire history, fire severity and woody encroachment on post-fire microbial communities will help inform future land management decisions that aid in preserving these important ecosystem services (van der Putten *et al.* 2013). Further, elucidating how soil chemistry and microbial communities are coupled is a critical step toward understanding how fire history and severity affect abiotic and biotic ecosystem attributes directly and indirectly. Studies that compare fire severities in grasslands are rare, and we were motivated to investigate how woody encroachment and fire suppression that potentially fuel greater fire intensities and burn severities may interact to affect abiotic and biotic soil attributes.

We acknowledge the limitations of our experimental design stemming from spatially and temporally confounding variables. By sampling plots within two watersheds rather than multiple replicate watersheds, we are confined to extrapolating our results from these watersheds. However, our experiments targeting soil chemistry and microbial community responses to encroachment on a watershed level (Q1) or to encroachment locally within a watershed (Q2) focused on encroachment but accounted for fire history effects differently. These experiments yielded consistent results that corroborate an existing knowledge base. In addition, the low and high fire severities differed in impacts, as we attempted to decouple history of fire suppression and encroachment, despite being constrained to the local characteristics of the local ecosystem.

It remains unclear whether different fire severities facilitate or suppress woody encroachment over time and whether they could be employed as a grassland restoration tool (Twidwell *et al.* 2016). Whether high-severity fire could be exploited as a restoration opportunity to encourage system transition from one state (encroached grassland) to another (native non-encroached prairie) remains an open question. Although we lack the long-term data to assess how our microbial community states progress along different trajectories, our data suggest that high-severity fire initiates a novel state that is distinct from either the grass- or shrub-dominated ones. Indeed, pyrodiversity may beget biodiversity on the landscape level in this system as predicted by the pyrodiversity-biodiversity hypothesis (Martin and Sapsis 1992; Jones and Tingley 2021). However, this was only true for the high-severity treatments; the low-severity fire at the local or landscape scale only minimally impacted abiotic and biotic system attributes, retaining communities that were distinct between watersheds (Q1) or local vegetation patches (Q2, Q3).

Soil chemistry

Woody encroached watershed that was protected from fire for 27 years at the time of our sampling differed in SOM, TotC and TotN accumulation compared to an annually burned, non-encroached watershed (Q1). This effect of fire suppression and the absence of aboveground biomass removal on soil C is consistent with other studies of woody encroachment increasing soil C accumulation (McKinley and Blair 2008; Barger *et al.* 2011; Connell, Nippert and Blair 2020) but contrasts others (Smith and Johnson 2003). Our casual observations that *C. drummondii* islands also include abundant understory grasses and some forbs (personal observation) suggest that C inputs from grasses may not be replaced but rather supplemented by shrub inputs over time.

Overall, recent low-severity fire had little impact on soil chemistry that was distinct between the watersheds and remained so after the low-severity fire treatment. Within the fire protected watershed, our comparison between *Cornus*-encroached and grass-dominated patches (Q2) indicated minimal differences attributable to vegetation. These results suggest that the watershed context—most likely reflecting differences in fire history—is a more important determinant of soil chemical attributes than the woody encroachment that is driven by fire exclusion. It is also of note that the *Cornus* shrubs likely established at different times, and thus vary in age, whereas the two watersheds have experienced 27 years of compounding effects of distinct fire histories. Our analyses also suggest that low-severity fires had only little impact on soil chemistry, a result of minimal heat penetration into the soil profile paralleling that of a warm, sunny day as indicated by our recorded heat sensor data. A notable exception was plant available P that increased after the low-severity burn. At KPBS, P is limiting (Collins-Johnson *et al.* 2010) and mineralization of plant available P after low-severity fires may be biologically important, particularly as the low-severity prescribed fires are most commonly employed early in the growing season. In contrast to the low-severity fire, the high-severity fire (Q3, Fig. 6) distinctly altered soil chemistry and increased soil NH_4^+, plant available P and pH regardless of woody encroachment.

While low-severity fires may not substantially impact abiotic soil attributes, high-severity fires clearly do. Burn severity directly links to heat penetration in soil (Smith, Cowan and

Fitzgerald 2016) and subsequent impacts of heat on the abiotic and biotic soil properties (Massman 2012; Reazin *et al.* 2016). Depending on fire intensity and burn severity, fires can lead to immediate loss of N and C through combustion. High surface temperatures during a severe burn can combust the ligno-cellulosic biomass, and large volumes of living or detrital biomass can transform into partially burned pyrogenic carbon (Keiluweit *et al.* 2010). This can result in smaller organic matter pools near the soil surface (Certini 2005). However, general fire impacts on soil C and N pools remain unresolved. A meta-analysis concluded that forest fires increase soil C and N storage (Johnson and Curtis 2001) due to charcoal sequestration and post-fire succession by N-fixing plants. In contrast, a later meta-analysis concluded that fires reduce soil C and N in temperate forest systems (Nave *et al.* 2011). Fire suppression in a grassland system was associated with the accumulation of SOM and nutrients (TotN and TotP; Fig. 1) in the absence of frequent fire that had resulted in woody encroachment (Q1), although we cannot rule out other, unmeasured watershed-specific drivers. Our data also highlight the loss of soil C as a result of recent high-severity fire regardless of the vegetation. Taken together, these data indicate system (encroached vs. non-encroached grassland) and burn severity dependencies that need to be considered when generalizing about abiotic responses to fire.

An alternative possibility to direct fire effects on soil chemistry is that biological uptake was simply higher following low-severity fire because of the high mortality in high-severity treatment (Figure S2, Supporting Information). We collected soil samples 28 days following burning, which is unlikely enough time for microbial uptake to make up the difference in Mehlich P and NH_4^+ in the high-severity fire treatments (Fig. 6). A meta-analysis by Wan, Hui and Luo (2001) concluded that fires substantially increased soil NH_4^+ and NO_3^- in the short term, but that NH_4^+ returned to pre-fire levels 1 year later and NO_3^- within 2 years, on average. In addition, fires immediately increase available P (Serrasolsas and Khanna 1995) due to conversion of organic P to orthophosphate in soil (Cade-Menun *et al.* 2000). Our results align with these findings, particularly so in the high-severity fire treatments, in which inorganic nutrient responses (Mehlich P, NH_4^+) were most obvious, regardless of the vegetation. In contrast, after low-severity fires, we observed a smaller post-fire increase in Mehlich P and a decrease in NH_4^+. In sum, bioavailable P consistently increased after fire, whereas N responses depended on fire severity. These results again highlight that the fire severity can affect the direction and magnitude of changes in soil chemistry.

Bacterial and fungal communities

Long-term fire suppression in watersheds that differed in their fire histories differed in woody encroachment and had distinct bacterial and fungal communities (Q1). Like soil chemistry, the community responses depended on burn severity and rarely on the vegetation within the encroached watershed. However, after a high-severity burn (Q3), bacterial and fungal richness and diversity decreased (Fig. 7), and communities shifted and diverged in both grass-dominated and shrub-encroached soils (Fig. 3). Following the low-severity burn, such bacterial community responses were not evident across the two watersheds or vegetation. However, low-severity burns increased bacterial richness and diversity regardless of fire history (Fig. 2) or vegetation (Fig. 4). In contrast, fungal richness and diversity consistently declined after both severity treatments, but the extent of decline following high-severity fires was more pronounced. These observed community responses to fire corroborate reviews (Dooley and Treseder 2012; Pressler, Moore and Francesca Cotrufo 2019) highlighting that fungal communities are generally more sensitive to fire than bacteria, with coinciding declines in fungal richness. Although both bacterial and fungal communities were distinct in the two watersheds that differed in fire histories and in woody encroachment, only fungal communities responded to a low-severity fire in these watersheds. The experimental high-severity fire, however, caused substantial losses of diversity and changed both bacterial and fungal communities compositionally.

Soil bacterial communities did not differ between grass-dominated areas and shrub islands. In contrast, the fungal communities were distinct within and beyond established shrubs before the fire and after low-severity fire (Q2). However, our data indicate that these communities were homogenized by the high-severity fire such that fungal communities were no longer distinct after fire either underneath or beyond established shrub islands (Fig. 3). Reazin *et al.* (2016) demonstrated that, in a Ponderosa pine forest, high-severity fires impacted fungal soil communities more substantially than low-severity fires. Our results extend these findings to show that high-severity fire not only impacted fungal communities more dramatically, but that it erased community differences between adjacent encroached and non-encroached landscape units. In other words, physically adjacent systems in distinct system states (grass vs. shrub—exemplifying hysteresis in this prairie system) inhabited by distinct fungal communities transitioned after high-severity fire to a system state characterized by non-distinct soil fungal communities. However, bacterial communities in soils within and beyond established shrubs were similar before fires and did not change after the low-severity fire. In contrast to the low-severity fire, high-severity fire resulted in a strong community response and the post-fire bacterial communities were indistinguishable regardless of where that high-severity fire had been applied. So, while both bacterial and fungal communities transitioned to a novel community state after the high-severity fire, bacterial communities were compositionally resistant to the effects of local vegetation and low-severity fire.

Overall, bacterial and fungal communities differed in their responses to fire, watershed fire history and fire severity. Bacterial richness and diversity consistently increased in response to low-severity fires, whereas fungal richness and diversity remain unchanged. We speculate that this was due to rapid post-fire dispersal of bacteria to the soil surface (Kobziar *et al.* 2018), as burning the top-most soil horizons may have wiped out a substantial proportion of the most abundant taxa/OTUs. This conclusion is consistent with the observed increase in bacterial diversity (*H'*) following the low-severity fire. For fungi, univariate measures of richness and diversity were responsive to fire history albeit less so to our experimental low-severity fire. Compositionally, however, fungal communities did respond to low-severity fire at both the local and watershed levels. With a combination of uni- and multi-variate measures of diversity and community composition, our results further corroborate evidence that fungi are sensitive to fire events in the short- and long-term (Dooley and Treseder 2012; Pressler, Moore and Francesca Cotrufo 2019) and more so than bacterial communities (Carson *et al.* 2019).

Taxon-specific responses to fire

Our analyses identified taxa that were associated with high-severity burns, including bacteria and fungi that were rare in both pre-fire and post-low-severity fire communities (e.g. fungal OTU44 *P. melinii*; bacterial OTUs 54 and 79 in the family Moraxellaceae). Importantly, our indicator taxon analyses highlighted the presence of pyrophilous Pyronemataceae (OTUs 7 and 10 putative *Anthracobia* sp. and *Pyronema* sp., respectively, as suggested by BLAST searches, even though the former was initially assigned to *Scutellinia* in the UNITE reference-based analyses). The most abundant fungal indicator taxon was *N. terricola* (Figure S6, Supporting Information), a species in a genus often associated with burned vegetation and characterized by fire-stimulated spores (Turner, Perkins and Fairfield 2001; Jacobson *et al.* 2004; Dettman, Jacobson and Taylor 2006). *Penicillium* spp. commonly occur after high-severity burns (Mikita-Barbato, Kelly and Tate 2015; Whitman *et al.* 2019; Hopkins, Semenova-Nelsen and Sikes 2020; Pulido-Chavez *et al.* 2021), although these fungi are more likely abundant opportunists and fast colonizers than fire adapted taxa *per se*. Such opportunists may be capable of utilizing fire-affected organic matter or cytoplasmic contents released from dead cells taking advantage of newly available nutrients in the soil matrix (Whitman *et al.* 2019).

Removal of aboveground vegetation and altered soil chemistry make for a harsh post-fire environment where microbes are potentially exposed to increased solar radiation and low soil moisture (Marcolin *et al.* 2019). An open question is to what extent species from local and regional pools vegetatively expand into fire-disturbed habitats from adjacent, less fire-exposed patches and/or deeper soil profiles or arrive and colonize by air. Although there is a long history of research on post-fire effects in ecosystems, there is a still debate on whether pyrophilic organisms are already present within the environment and respond to physical or chemical fire-generated cues or whether pyrophilic organisms disperse into the fire-disturbed environments anew (Kobziar *et al.* 2018; Mims and Mims 2004; Moore *et al.* 2021). Whitman *et al.* (2019) proposed a conceptual model of fire-responsive microbial traits analogous to plant traits (e.g. heat surviving, opportunistic growth and affinity for post-fire environment). Several such microbial traits have been tested for fire-responsive fungi. Resting structures and spores may be heat (*Rhizopogon*, Bruns, Hale and Nguyen 2019) or chemically stimulated by fire (*Neurospora*, Dettman, Jacobson and Taylor 2006), residing in the soil for years between fire events (Bruns, Hale and Nguyen 2009; Peay, Garbelotto and Bruns 2009; Glassman *et al.* 2016). Fire-responsive bacteria such as species within *Bacillus* and *Clostridium* can also produce spores that resist temperatures above 100°C (Theodorou and Bowen 1982). Rhizosphere bacteria may be stimulated by fire to some extent and aid in post-fire nitrogen cycling (Cobo-Díaz *et al.* 2015; Fernández-González *et al.* 2017).

We posit that there is less resistance to high-severity fires than low-severity fires within the soil profile that is directly impacted by combustion during a high-severity burn or exposed to extended periods of high temperatures. Thus, communities there must largely re-establish. Soil community negative responses can be highlighted by the direct mortality of many saprobes as the substrates that they inhabit combust (Hopkins, Semenova-Nelsen and Sikes 2020) as well as the indirect mortality of plant-associated microbes (e.g. mycorrhizal fungi, fungal and bacterial endophytes) as a result of fire-induced mortality or altered physiology of their hosts. Fire may reduce competition in the soil (El-Abyad and Webster 1968a), trigger spore germination (El-Abyad and Webster 1968b), raise the pH (Certini 2005) and produce a pulse of available nutrients (Kutiel and Naveh 1987). Any combination of these factors may alter relative competitiveness in the resident communities and stimulate organisms that respond positively and proliferate across fire-impacted landscapes. High-severity fires may be unique in that they may open niches for opportunistic taxa that a low-severity fire would not (Figure S6, Supporting Information), and these taxa may thrive in post-fire grasslands (Yang *et al.* 2020) or forests (Fujimura *et al.* 2005; Hughes *et al.* 2020).

CONCLUSION

Fires can dramatically influence both biotic and abiotic ecosystem attributes (Certini 2005; Pressler, Moore and Francesca Cotrufo 2019; Certini *et al.* 2021). Fire frequencies and severities are currently changing because of changing climate and many anthropogenic drivers (Andela *et al.* 2017; Pellegrini *et al.* 2018). Thus, it is crucial to understand how ecosystems respond to changing fire regimes. Our study demonstrates that soil chemistry as well as fungal and bacterial communities respond to high-severity burns regardless of woody encroachment in a grassland system. In contrast, low-severity fires had divergent effects on soil nutrients as well as bacterial and fungal communities and depended on whether encroaching shrubs were present. Contrasting fire histories between the two watersheds suggest a stronger effect of fire suppression on soil chemistry and microbial communities than encroaching vegetation within the fire-protected watershed. Historically, North American prairies burned frequently (Knapp *et al.* 1998), but extensive habitat loss, fragmentation and changes in fire management have resulted in substantial losses of prairie systems (Samson and Knopf 1994; Leach and Givnish 1996). In the context of tallgrass prairie ecosystems in the central United States, our results suggest that a high-severity fire may lead to novel system states, the trajectory of which remains unknown. Since the watershed effects, including a history of fire suppression, were a stronger influence on microbial communities than local encroachment, the importance of frequent fires to prevent woody encroachment is supported and extreme interventions like high-severity fires require further investigation.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

AUTHOR CONTRIBUTIONS

We use this to justify author invitations. LM—coordinated field sampling, generated soil chemistry and microbial community data and edited final manuscript; MRK—analysed soil chemistry and microbial community data, wrote the manuscript and edited the final manuscript; SF—established the field experiment, sampled the field experiment, processed acquired field samples and edited the final manuscript, CR—established the field experiment, sampled the field experiment, processed acquired field samples, generated microbial community data and edited the final manuscript; LZ—conceptualized and designed the experiment and edited the final manuscript; AJ—conceptualized and designed the experiment, established the field experiment, sampled the field experiment, processed acquired field samples, wrote the manuscript and edited the final manuscript.

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Conflict of interest. None declared.

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