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Fire affects the taxonomic and functional composition of soil microbial communities, with cascading effects on grassland ecosystem functioning

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Abstract

Fire is a crucial event regulating the structure and functioning of many ecosystems. Yet few studies have focused on how fire affects taxonomic and functional diversities of soil microbial communities, along with changes in plant communities and soil carbon (C) and nitrogen (N) dynamics. Here, we analyze these effects in a grassland ecosystem 9 months after an experimental fire at the Jasper Ridge Global Change Experiment site in California, USA. Fire altered soil microbial communities considerably, with community assembly process analysis showing that environmental selection pressure was higher in burned sites. However, a small subset of highly connected taxa was able to withstand the disturbance. In addition, fire decreased the relative abundances of most functional genes associated with C degradation and N cycling, implicating a slowdown of microbial processes linked to soil C and N dynamics. In contrast, fire stimulated above- and belowground plant growth, likely enhancing plant–microbe competition for soil inorganic N, which was reduced by a factor of about 2. To synthesize those findings, we performed structural equation modeling, which showed that plants but not microbial communities were responsible for significantly higher soil respiration rates in burned sites. Together, our results demonstrate that fire ‘reboots’ the grassland ecosystem by differentially regulating plant and soil microbial communities, leading to significant changes in soil C and N dynamics.

KEYWORDS: Californian grasslands, climate change, fire, GeoChip, high-throughput sequencing, microbial communities

1 INTRODUCTION

Anthropogenic activities, and in particular changes in land use and management, have considerably altered fire regimes (Andersson, Michelsen, Jensen, & Kjøller, 2004). Climate change is likely to also affect fire regimes because antecedent wet periods could provide herbaceous fuels for fire, while drought and warming could extend weather patterns that are conducive to fire (Bowman et al., 2009). As a consequence, recent years have witnessed increased fire frequencies in many areas, and it is anticipated that fire frequency and intensity will further increase in the coming century (Westerling, Hidalgo, Cayan, & Swetnam, 2006). It is likely that fire in California would burn more rapidly, with higher intensity, under warmer climate scenarios (Fried, Torn, & Mills, 2004).

Fire affects plant diversity by increasing resource availability for subordinate and early-successional species (Pickett, White, & Brokaw, 1985). Fire also has a short-term stimulatory effect on N availability to plants but a long-term negative effect due to increased N losses (Reich, Peterson, Wedin, & Wrage, 2001). Fire can stimulate fine root production and aboveground net primary production in the first postfire growing season in grasslands (Docherty, Balser, Bohannan, & Gutknecht, 2011; Strong, Johnson, Chiariello, & Field, 2017). Changes in the aboveground communities could indirectly affect the belowground systems via altering nutrient inputs, increasing solar warming and surface soil temperatures, and changing evapotranspiration and soil moisture (Hart, DeLuca, Newman, MacKenzie, & Boyle, 2005; Strong et al., 2017). Fire can also induce higher greenhouse gases emissions, including CO_2 (Strong et al., 2017) and N_2O (Niboyet et al., 2011). Responses of soil C dynamics to fire vary between ecosystems. For instance, soil respiration was stimulated shortly post fire and then decreased in a tropical savanna woodland (Andersson et al., 2004) and a permafrost forest (Tas et al., 2014), but CO_2 efflux rates decreased post fire in a ponderosa pine forest (Sullivan et al., 2010) and in an arid grassland (Vargas et al., 2012). In a grassland ecosystem, three mechanisms—increases in soil temperature, fine root production, and aboveground net primary production—were shown to explain changes in soil respiration rates postfire (Strong et al., 2017).

Fire plays an important role in structuring and altering underground microbial communities. Fire can directly kill microbes by heating the soil upper layer and indirectly affect microbial community composition by altering plant community composition and plant-driven organic matter inputs to soil (Docherty et al., 2011; Hart et al., 2005), as well as soil environmental conditions. In boreal forests, fire decreased microbial biomass and dissolved organic C in surface organic horizons 5 years postfire (Waldrop & Harden, 2008). In contrast, fire stimulated microbial growth by increasing soil ammonium (NH_4^+) and dissolved organic C concentrations by 20%–52% in a tropical savannah woodland (Andersson et al., 2004). However, microbial N immobilization and plant N uptake were reduced by fire. In grasslands, enzyme activities related to C degradation and P cycling decreased, the effect being mild in the first growing season post fire, higher in the second growing season post fire but subsidizing in the third growing season post fire (Gutknecht, Henry, & Balser, 2010). In contrast, microbial enzyme activities related to organic N degradation have been shown to either decrease (Gutknecht et al., 2010) or increase (Ajwa, Dell, & Rice, 1999), post fire, likely due to site-specific differences. As fire could strongly affect soil nutrients, responses of microbial functional groups to pulse increases of nutrient availability could cause long-term changes in ecosystem. For example, fire significantly decreased soil microbial biomass and changed compositions of nitrogen-fixing and ammonia-oxidizing communities in a mixed conifer forest, linking to fire-related increases in soil NH_4^+ or soil pH (Yeager, Northup, Grow, Barns, & Kuske, 2005). This can change nitrification rates, NO and N_2O flux in postfire ecosystems (Serça et al., 1998). Overall, some inconsistencies among fire effects on soil N processes have been reported, which reflect differences in soil type, fire intensity, vegetation type, and the timing of measurements post fire.

Microbe-mediated soil bioprocesses are considered to be one of the important drivers of ecosystem recovery from fire (Neary, Klopatek, DeBano, & Ffolliott, 1999). Although many studies have investigated the responses of below- and aboveground communities to fire in grasslands, studies examining the fire effects on soil microbes have largely focused on microbial population sizes and microbial activity (Ajwa et al., 1999; Andersson et al., 2004; Gutknecht et al., 2010; Waldrop & Harden, 2008; Yeager et al., 2005). In contrast, how fire affects the total soil microbial community composition largely remains to be studied in grassland ecosystems, and it is unclear which assembly processes control postfire microbial community dynamics. Moreover, it is needed to analyze fire effects not only on the taxonomic diversity of soil microbial community, but also on its functional diversity, particularly the range of microbial functional groups linked to soil C and N dynamics is required to expand our understanding of the effects of fire on soil function in grassland ecosystems.

Here, we studied fire effects in an annual Mediterranean grassland in California, USA, as part of the long-term Jasper Ridge Global Change Experiment (JRGCE) (Henry, Juarez, Field, & Vitousek, 2005). In particular, we examined fire effects on the soil microbial community 9 months after the prescribed burn in July 2011. We used bacterial *16S rRNA* gene amplicon sequencing (Illumina MiSeq) and functional gene array (GeoChip 4.6) analyses to examine the effects of fire on the taxonomic and functional diversities of soil microbial communities. These techniques allow for high-resolution profiling of community composition for both facets of microbial diversity. We also examined whether the fire would have cascading effects on ecosystem functioning such as soil CO_2 efflux.

Given that fire typically increases soil C and N inputs, we hypothesized that there would be a shift in microbial taxonomic composition, and more particularly that functional genes associated with labile C degradation would be stimulated, leading to higher soil CO_2 efflux. Since previous results at our study site showed that fire significantly increased soil NH_4^+ concentrations (Docherty et al., 2011) in the first growing season post 2003 fire, and soil N_2O emission (Niboyet et al., 2011) in the second and third growing seasons after a 2003 fire, we also hypothesized that functional microbial groups associated with N cycling would be stimulated.

2 MATERIALS AND METHODS

2.1 Site description

The Californian annual grassland ecosystem experiences a Mediterranean-type climate, in which the growing season is cool and wet from November to May, and the rest of the year is hot and dry. The plant community comprises non-native annual grasses, perennial native grasses, native annual forbs, and non-native forbs (Henry et al., 2005). Soil mainly consists of weathered alluvium, which is a fine and mixed Typic Haploxeralf. This experiment was initiated in October 1998 at the JRGCE site located in central California, USA (37^{o}24′N, 122^{o}13′W). Initially, the JRGCE was designed to assess grassland ecosystem responses to single and multiple components of global changes, including elevated CO_2, warming, N deposition, and enhanced precipitation. To this end, each 2-m diameter circular plot at the JRGCE was equally divided into four quadrants (i.e., subplots) of 0.78 m^2. N deposition and enhanced precipitation treatments were arranged at the quadrant level and the elevated CO_2 and warming treatments were arranged at the plot level in a full factorial design. In the summer of 2003, an accidental wildfire burned two of the eight blocks of the experiment (i.e. 32 of the 128 subplots). In the summer of 2011, a controlled fire was applied to four of the eight blocks (i.e. 64 of the 128 subplots including the subplots that were already burned in 2003). Soil samples were collected on 26 and 27 April 2012, that is, 9 months postfire and 14 years after the experiment began. We collected one soil core with 5 cm diameter × 7 cm deep per quadrant to generate each of 64 unburned and 64 burned samples. We thoroughly mixed each sample and sieved soil through a 2 mm mesh. Those soil samples were stored at −80°C prior to DNA extraction or at −20°C prior to soil geochemical measurements.

2.2 DNA extraction, purification, and quantification

DNA was extracted from 5 g of soil samples with a freeze-grinding mechanical lysis method (Zhou, Bruns, & Tiedje, 1996). DNA was purified by gel electrophoresis with a 0.5% low melting point agarose, then extracted with phenol and chloroform and finally precipitated with butanol. Using a Nanodrop (Nanodrop Technologies Inc.), DNA quality was assessed by ratios of light absorbance at A_{260}/A_{280} (>1.8) and A_{260}/A_{230} (>1.7). The final DNA concentration was quantified using a PicoGreen® method using a FLUOstar Optima microplate reader (BMG Labtech; Liu et al., 2015).

2.3 16S rRNA amplicon sequencing and raw data processing

A polymerase chain reaction (PCR) amplification targeting the V4 hypervariable region of 16S rRNA genes was carried out to examine the bacterial community diversity, using the primers F515 (5′-GTGCCAGCMGCCGCGG-3′) and R806 (3′-TAATCTWTGGGVHCATCAG-5′). The PCR products were sequenced by 2 × 250 bp paired-end sequencing with MiSeq instrument (Illumina). Raw sequence data were processed on the Galaxy platform (http://zhoulab5.rccc.ou.edu:8080). The paired-end raw sequences were demultiplexed to remove PhiX sequences and then sorted to appropriate samples based on paired barcodes. These sequences were then trimmed with Btrim based on quality scores and paired-end reads were combined with FLASH into full-length sequences (Magoc & Salzberg, 2011). Sequences less than 200 bp or containing ambiguous bases were removed and chimeric sequences were discarded based on prediction by Uchime using the reference database mode. Operational taxonomic units (OTUs) were clustered at 0.97 identity level by UCLUST (Edgar, 2010; Edgar, Haas, Clemente, Quince, & Knight, 2011). Taxonomic annotation was assigned to OTUs based on representative sequences with RDP's 16S Classifier. The number of sequences retrieved from each sample might differ, and this reflects different efficiency of the sequencing process rather than actual biological variation (Weiss et al., 2017). We thus standardized the number of sequences across samples.

2.4 GeoChip hybridization and raw data processing

To study functional genes of bacterial, archaeal, and fungal communities, DNA hybridization with GeoChip 4.6 was carried out as previously described (Yang et al., 2013). Microbial DNA extracted from soil was labeled with Cy-3 fluorescent dye using random priming method, and then purified with a DNA purification kit (Qiagen). After drying at 45°C with SpeedVac (ThermoSavant), labeled DNA was hybridized to GeoChip 4.6 at 42°C for 16 hr in a MAUI hybridization system (BioMicro). After washing the slides with buffers provided by NimbleGen, the hybridized DNA was scanned using a NimbleGen MS 200 Microarray Scanner (Roche).

Raw GeoChip data were processed as follows: (a) remove the spots with a signal-to-noise ratio less than 2.0; (b) perform log-transformation on the signal intensity for each gene and divide them by the mean intensity in the sample; and (c) remove genes detected only in one out of the four replicates from the same treatment.

2.5 Soil abiotic measurements

To characterize the microclimate of the soil where samples were taken, soil temperature at the depth of 2 cm was measured hourly during 2012 with in situ thermocouples. The average values measured during April 2012 (the month when soils were sampled) were calculated. One soil core (5 cm diameter × 7 cm deep) per quadrant was sampled and stored at −20°C prior to soil abiotic measurements. Soil moisture was measured by drying 10 g of freshly collected soil at 105°C for 1 week. Soil pH was measured using 5 g of soil dissolved in distilled water. Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were measured by suspending 5 g of soil in a 2 M KCl solution, filtering the extracts, and analyzing them colorimetrically using a SEAL Automated Segmented Flow analyzer in the Loyola University, Chicago, IL, USA. Soil CO_2 efflux of each sample was measured three times by a closed-chamber method in April 2012 as previously described (Strong et al., 2017), where a LiCOR LI-6400 portable photosynthesis system and LI6400-09 Soil Flux Chamber (LiCOR, Lincoln) were used.

2.6 Vegetation variable measurements

Aboveground plant biomass was collected from 141 cm^2 of each quadrant on the sampling in April 2012. Individual plant species were separated into functional groups (annual grasses, perennial grasses, annual forbs, perennial forbs, and litter), and the biomass was oven-dried at 70°C and weighed. Root biomass was estimated by separating roots from two soil cores (2.5 cm diameter by 15 cm depth) from the same area of the aboveground biomass collection, and the root biomass was oven-dried at 70°C and weighed.

2.7 Statistical analyses

Unpaired *t* tests were used to determine fire effects on relative abundances of microbial taxa, functional genes and soil and vegetation variables. *p* Values were adjusted by the Benjamini–Hochberg method with sequentially modified Bonferroni correction, using the p.adjust function in the vegan package in R version 3.5.0. Permutational multivariate ANOVA was implemented to rank global change drivers of the composition of environmental variables, using adonis function in the vegan package. ANOVA was performed to rank global change drivers of each environmental variable, using aov function in the vegan package. Three nonparametric dissimilarity tests (multi-response permutation procedure [MRPP], analysis of similarities [ANOSIM] and nonparametric multivariate analysis of variance [adonis] in the vegan package in R) were used to compare differences of microbial community composition. Shannon Index (H') was used to calculate the α-diversity of the microbial community in the unburned and burned plots, using the alpha.g function in the ieggr package in R.

Microbial community assembly could be shaped by interspecies competition for resources and cooperative interactions (Zelezniak et al., 2015). Metabolically interdependent microbial groups are strongly coupled through mutualistic interactions, which support species growth; thus these groups form persistent sub-networks within the soil microbial communities (Zelezniak et al., 2015). Such high degree of connectivity among keystone taxa can dampen soil microbial compositional changes in response to environmental changes and disturbances (Herren & McMahon, 2018; Zelezniak et al., 2015). Therefore, we used linear mixed model to examine whether the connectivity among keystone taxa, measured by cohesion metrics, could explain microbial compositional changes. Cohesion metrics measure the instantaneous connectivity of a microbial community and contain two metrics showing the strength of positive and negative relationships between taxa. Bray–Curtis dissimilarity was used to measure community compositional changes at OTU level. The regression analysis using the most highly connected taxa was conducted to examine the relationship between Bray–Curtis dissimilarity and cohesion metrics. The R^2 of each model was recorded. Highly connected taxa would be better predictors of the whole bacterial community than randomly selected taxa since the former has disproportionate influence in determining bacterial community dynamics. Regression analyses using random subsets of taxa were repeated 500 times to generate a distribution of model R^2. We compared the R^2 value of a model using the most highly connected taxa with that of models using random taxa, if the former is higher than the 95th percentile of the latter, then the model using the most highly connected taxa was considered to be significantly different from the model using random taxa, suggesting that interactions among keystone taxa indeed have an important role regarding compositional changes of the bacterial community. In addition, we used the pnull function in the ieggr package in R to calculate the β-nearest taxon index (βNTI), which measures the degree to which microbial community composition is governed by environmental filtering based on OTUs' ecological niches (Stegen et al., 2013).

Using the psem function in the piecewiseSEM package in R, structural equation models (SEM) were also fitted to calculate the direct and indirect effects of soil variables, soil microbial community composition, and plant biomass on soil CO_2 efflux rates. Model fits were measured by a nonsignificant Shipley's test of d-separation, with Fisher's $p > .05$ indicating that no paths were missing, and low Akaike information criterion being used to choose among competing models (Jing et al., 2015). The first principal components (PCo1) of microbial taxonomic community were used to represent the microbial community composition, using the pco function in the vegan package in R.

3 RESULTS

The impact of fire on the soil bacterial community did not vary largely across different levels of CO_2, nitrogen deposition, warming, and precipitation (Table S1). Furthermore, of over 26 possible interactive effects tested between fire and other global climate changes, only one was statistically significant—the interaction between fire and nitrogen deposition on soil nitrate concentration

(Tables S2 and S3). Since the responses to fire across the 16 factorial combinations of CO_2, nitrogen deposition, warming, and precipitation (n = 64) were similar to those for fire as a sole treatment (n = 4; Table S1), data were pooled across all the other treatment levels following a previous example (Reich et al., 2006). The n = 64 samples increased the statistical power and identified trends valid across the range of climate change scenarios tested at the Jasper Ridge Global Change Experiment.

3.1 Changes of taxonomic composition of bacterial communities by fire

A total of 8,882,825 sequences were obtained, ranging from 29,275 to 92,183 reads per sample. After resampling with 29,275 sequences per sample, 87,275 OTUs were obtained. Singletons were removed, which might represent inherently unreplicated data possibly resulting from sequencing errors (Dickie, 2010), leading to a total of 64,515 OTUs. Using three nonparametric statistical analyses (ANOSIM, adonis, and MRPP), we found that fire significantly changed taxonomic composition ($p \leq .003$ for all three methods; Table 1). However, the α-diversity of the bacterial community, based on Shannon index, was similar between the unburned (8.16 ± 0.02) and burned (8.24 ± 0.02) sites ($p = .270$). About 4,503 unique OTUs were detected in unburned plots, and 6,744 unique OTUs in burned plots, while 53,268 OTUs were found in both plots (Figure S1a). The relative abundance of *Proteobacteria* was significantly higher in burned plots ($p = .047$), while the relative abundances of *Planctomycetes* ($p = .047$), *OD1* ($p = .009$), *Chlorobi* ($p = .009$), *OP11* ($p = .007$), and *Spirochetes* ($p = .006$) were significantly lower in burned plots (Figure S1b). At finer taxonomic levels, relative abundances of 19 genera (such as *Agromyces*, *Ferruginibacter,* and *Pasteuria*) were significantly decreased, whereas those of 16 genera (such as *Actinoplanes*, *Bdellovibrio,* and *Flavisolibacter*) were significantly increased (Table S4).

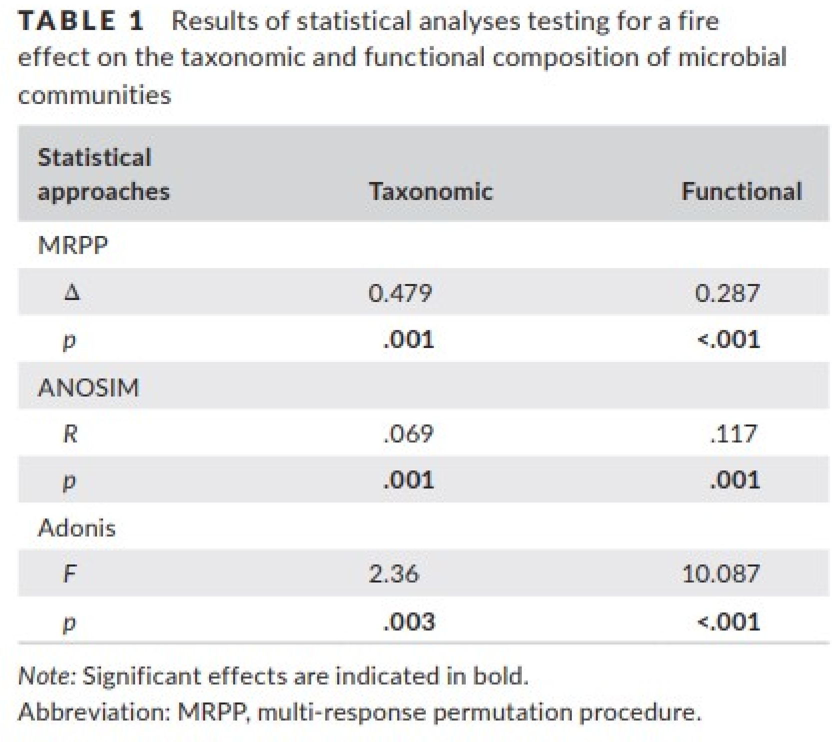

TABLE 1 Results of statistical analyses testing for a fire effect on the taxonomic and functional composition of microbial communities

Statistical approaches	Taxonomic	Functional
MRPP		
Δ	0.479	0.287
p	**.001**	**<.001**
ANOSIM		
R	.069	.117
p	**.001**	**.001**
Adonis		
F	2.36	10.087
p	**.003**	**<.001**

Note: Significant effects are indicated in bold.
Abbreviation: MRPP, multi-response permutation procedure.

Cohesion metrics were significant predictors of compositional changes in burned sites (p = .017 and p < .001; Table S5), with both the negative and positive cohesion metrics related to the compositional changes (p < .001; Figure S2). The R^2 values of models using the most highly connected taxa remained unchanged when the least-connected taxa were removed, whereas the R^2 values of models containing random subsets of taxa decreased when more taxa were removed (Figure 1a). The explanatory power of these models increased to 13% when a small subset (46) of highly connected taxa was used to calculate the cohesion metrics, suggesting that biotic interactions among taxa could partially explain the community compositional change. These keystone taxa included 13 genera from *Proteobacteria* (such as *Rudaea*, *Bauldia,* and *Microvirga*), nine genera from *Acidobacteria* (*Gp1-Gp7*, *Gp16,* and *Gp17*), six genera from *Actinobacteria* (such as *Rugosimonospora*, *Catenulispora,* and *Agromyces*), six genera from *Bacteroidetes* (such as *Terrimonas*, *Adhaeribacter* and *Ferruginibacter*), four genera from *Planctomycetes* (*Gemmata*, *Planctomyces*, *Singulisphaera,* and *Isosphaera*), and seven genera from *Armatimonadetes*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia,* and *WS3* (Table S6).

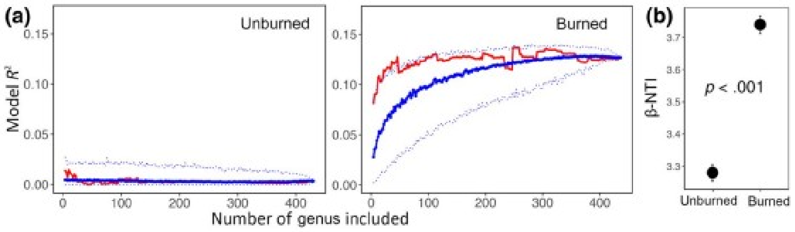

FIGURE 1 The panel on the left (a) indicates relationships between the adjusted model R^2 values for the Bray–Curtis dissimilarity versus cohesion regression analysis according to the number of taxa excluded from cohesion calculations under unburned or burned conditions. For each number of taxa on the *X*-axis, cohesion values were calculated for the most connected taxa (red lines) and for a random subset of taxa (blue lines). The solid blue lines show the median-adjusted model R^2 for randomly selected subsets, while the dotted blue lines give the 5% and 95% intervals. When 46 genera were used to calculate cohesion under burned conditions, models using the most highly connected taxa had significantly higher model R^2 than models using random taxa, suggesting that biotic interactions among taxa could partially explain the community compositional changes post fire. The panel on the right (b) indicates β-nearest taxon index (β-NTI) in unburned and burned sites

In burned sites, the models with highly connected taxa showed significantly higher performance than those with randomly selected taxa (Figure 1a). As the negative cohesion was a better predictor of compositional changes of the bacterial community than positive cohesion (Figure S2), we focused on 46 genera showing the strongest negative associations with others. Among them, seven genera of *Gp5* ($p = .039$), *Agromyces* ($p = .010$), *Ferruginibacter* ($p = .039$), *Pasteuria* ($p = .001$), *Planctomyces* ($p = .001$), *Isosphaera* ($p = .041$), and *Verrucomicrobium* ($p < .001$) decreased in relative abundances compared to control (Figure S3). Many of those highly connected genera had low relative abundances. For example, *Rudaea* was the ninth most connected genus but its relative abundance ranked 144th among all the genera, with a mean relative abundance across all treatments of 0.03% (Table S6). In unburned sites, the explanatory power of models constructed using either randomly selected or most connected taxa remained very low (<2%; Figure 1a), suggesting that there was no keystone taxa effect in the unburned sites.

The βNTI, indicating the degree to which bacterial community composition is governed by environmental filtering, increased from 3.28 ± 0.025 in unburned plots to 3.74 ± 0.027 in burned plots ($p < .001$; Figure 1b), suggesting that the bacterial community was more strongly governed by environmental filtering in burned plots.

3.2 Changes in microbial functional potential by fire

A total of 68,695 microbial functional genes were detected by GeoChip. Fire caused significant changes in the overall functional composition of soil microbial communities according to the three nonparametric statistical analyses conducted ($p < .001$; Table 1) and significantly decreased microbial functional α-diversity.

For functional genes associated with C degradation, the signals detected (hereafter called relative abundances) for 16 relevant genes all declined in response to fire (see Figure 2). In contrast, only *camDCAB* had higher abundances for burned compared to unburned plots. For methane cycling, the abundances of the *mcrA* gene related to methanogenesis and *mmoX* genes related to methane oxidation decreased in burned sites (Figure S4). Other methane-oxidizing genes (*pmoA* and *hdrB*) remained unchanged.

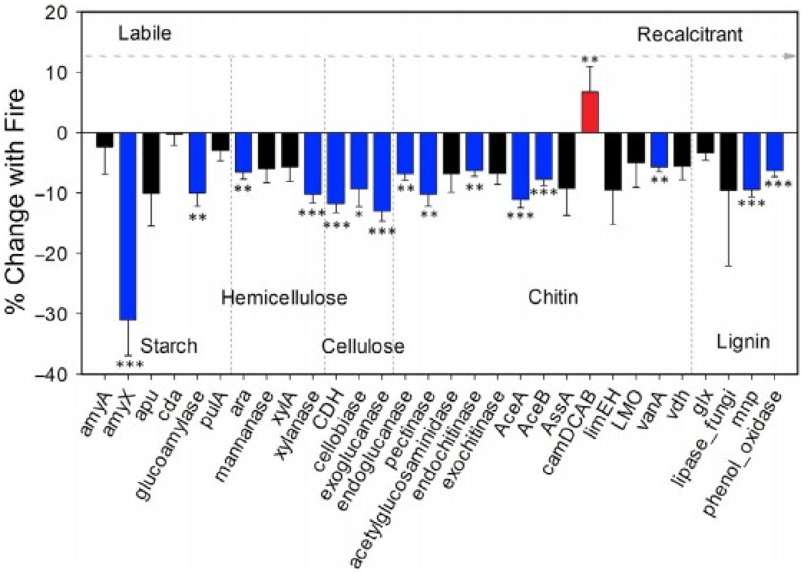

FIGURE 2 Percentage changes in relative abundances of C degradation genes induced by fire, calculated as: 100 × ((mean value for burned samples/mean value for unburned samples) − 1). The complexity of C is arranged from labile (on the left) to recalcitrant (on the right). Mean values and standard errors are presented (n = 64). Significant negative changes in the relative abundances of the genes by fire are indicated in blue, and significant positive changes in the relative abundances of the genes by fire are indicated in red. Asterisks indicate significant differences: $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .005$. p Values were adjusted by the Benjamini–Hochberg method using sequentially modified Bonferroni correction

Similar to C-cycling genes, a number of N-cycling genes also declined in response to fire (see Figure 3). In contrast, the abundances of the two genes *nifH* and *amoA* were not significantly altered by fire (Figure 3). The P-cycling genes encoding phytase, polyphosphate kinase (*ppk*), and exopolyphosphatase (*ppx*) also decreased in response to fire (Figure S5).

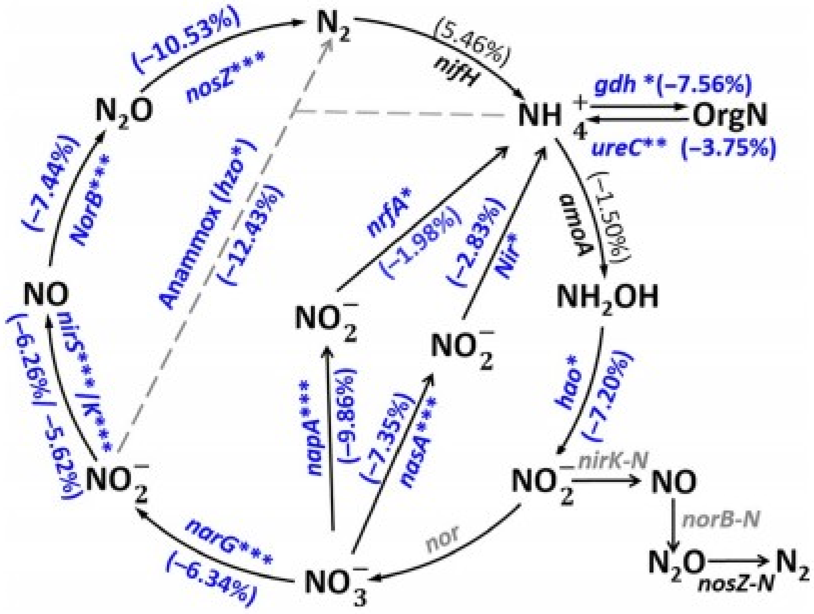

FIGURE 3 Percent changes in relative abundances of N-cycling genes induced by fire, calculated as: 100 × ((mean value in burned samples/mean value in unburned samples) − 1). Average signal intensities of the genes in blue were significantly decreased by fire. Average signal intensities of the genes in black were not significantly altered by fire. Genes in gray were not targeted by GeoChip. Asterisks indicate significant differences: *$p < .05$; **$p < .01$; ***$p < .005$. p Values were adjusted by the Benjamini–Hochberg method using sequentially modified Bonferroni correction

3.3 Influence of fire on soil and vegetation variables

After 9 months postfire, aboveground plant biomass (+25.8%; $p = .034$) and belowground biomass increased (+24.6%; $p = .080$) but litter mass decreased (−86.6%; $p < .001$; Table S7). Soil temperature also increased (+1°C; $p < .001$) (Table S7). Soil NO_3^- decreased from 346 to 189 mg/L ($p = .029$), and soil NH_4^+ decreased from 834 to 389 mg/L ($p = .034$; Table S7). Soil CO_2 efflux significantly increased in the burned plots (+37.1%; $p < .001$; Table S7). Other soil variables such as soil moisture and pH remained unchanged by fire (Table S7).

3.4 Drivers of changes in soil functioning and bacterial communities

In the unburned sites, soil CO_2 efflux was correlated with root biomass ($\beta = .436$; $p = .003$) and to a similar extent with bacterial community composition ($\beta = .421$; $p = .006$; Figure 4a). In contrast, soil CO_2 efflux in burned sites was only and strongly correlated with root biomass ($\beta = .936$; p

= .044; Figure 4b). In these burned sites, bacterial community composition was related to aboveground plant biomass (β = .205; *p* = .011) and soil moisture (β = .171; *p* = .043), though soil pH (β = 0.610; *p* < .001) had stronger effects on microbial community (Figure 4b).

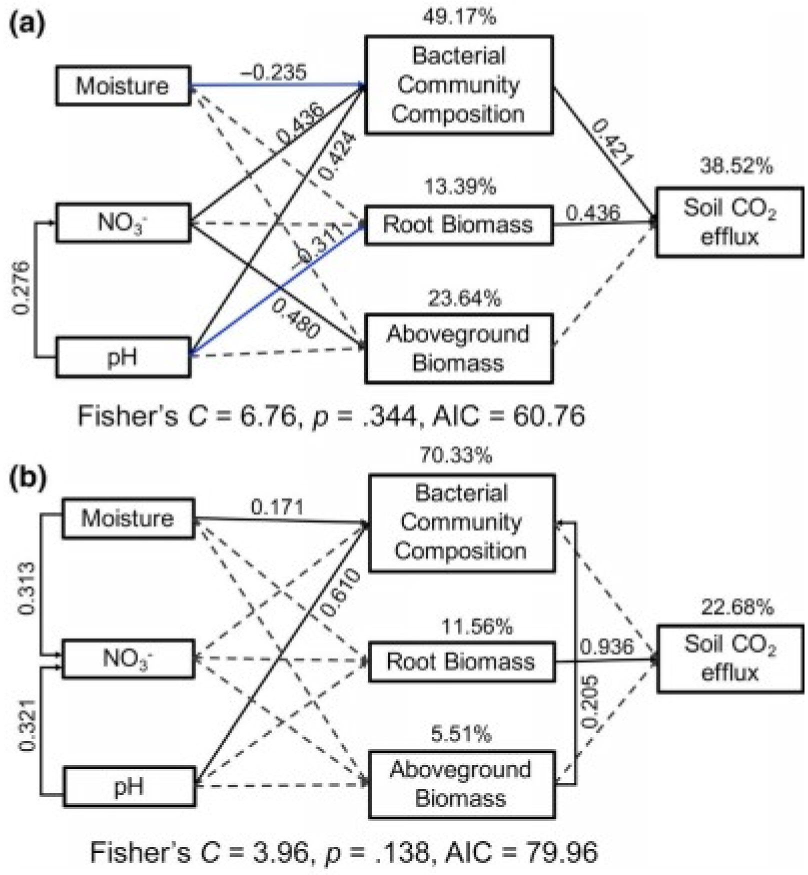

FIGURE 4 Structural equation models using soil variables, bacterial community composition, and above- and belowground plant biomass as predictors of soil CO_2 efflux rate for (a) unburned and (b) burned plots. Solid lines represent significant paths (*p* < .05, piecewise SEM), and dashed lines represent insignificant paths (*p* > .05, piecewise SEM). Blue arrows represent negative paths, and black arrows represent positive paths. NO_3^-, soil nitrate concentration; bacterial community composition, the first principal components (PCo1) of the principal coordinate analysis of soil bacterial community. Overall fit of piecewise SEM was evaluated using Shipley's test of d-separation, with Fisher's *C* statistics (if *p* > .05, then no paths are missing, and the model is a good fit) and Akaike information criterion (AIC). For each variable, the percentage of the variance explained by the model is indicated next to the box corresponding to this variable

4 DISCUSSION

Fire significantly altered the taxonomic composition of the bacterial community. The change could be predicted by cohesion metrics revealing the existence of ‘keystone’ taxa, which exert disproportionate influence on community composition through strong biotic interactions (Herren & McMahon, 2018). Biotic interactions can include metabolite exchanges among taxa, which enhance self-reinforcing modules within microbial communities that can stabilize community composition (Levy & Borenstein, 2013). More particularly, stronger negative cohesion was related to lower levels of compositional change in the burned sites (Table S5). Those strong negative cohesion values represented the negative feedback loops formed by biotic interactions among taxa, which had an important role in stabilizing community composition and mitigating the effects of fire. Similar results were reported for other environmental disturbances (Coyte, Schluter, & Foster, 2015; Konopka, Lindemann, & Fredrickson, 2015). This is also consistent with recent studies reporting that negative interactions among taxa, such as competition and predation, prevail in microbial communities (Herren & McMahon, 2018). For instance, a previous study focusing on bacterial strains isolated from a rainwater pool showed that negative interactions accounted for a great majority of pairwise taxa interactions, owing to potential resource competition among microbes (Foster & Bell, 2012). Since microbial diversity is typically high, investing in positive interactions often specific to a few other species could bring little evolutionary benefit, which may explain the dominance of negative interactions (Foster & Bell, 2012).

Higher βNTI values in burned sites indicated more phylogenetically clustered bacterial assemblages than expected by chance and suggested that fire increased the relative influence of deterministic environmental filtering for shaping bacterial communities (Yang et al., 2017). This result leads us to propose an explanation to the observed decreased microbial functional potential, especially those related to C degradation and N cycling. As fire stimulated above- and belowground plant growth, plant–bacterial competition for soil inorganic N might be enhanced, possibly imposing higher deterministic environmental filtering on bacterial communities. This would explain the higher βNTI values and is consistent with previous works on microbial assemblages in salt marsh, desert, agricultural fields, aquatic, and forest ecosystems (Caruso et al., 2011; Dini-Andreote, Stegen, van Elsas, & Salles, 2015; Mendes, Kuramae, Navarrete, van Veen, & Tsai, 2014; Wang et al., 2013; Yang et al., 2017). In particular, Jurburg et al. (2017) reported that a heat disturbance imposed a strong selective pressure on the community composition of active soil bacteria. In this case, phylogenetic turnover patterns indicated that the community experienced stronger deterministic selection during recovery after heat shock. Overall, significantly increased βNTI values suggested higher deterministic environmental filtering on soil bacterial communities following fire and provided an insight into the mechanisms structuring community composition.

In sharp contrast to higher above- and belowground plant biomass in response to fire (Table S7), most of the microbial genes related to C degradation were significantly decreased (Figure 2). Among all the 16,326 C-degradation genes detected in our study, 1.9% were archaeal genes, 14.7% were fungal genes, and 83.3% were bacterial genes. Archaeal, fungal, and bacterial C degradation genes were affected by fire to similar extent, approximately one-third of each category being significantly changed. Fire largely consumed the standing dead plant biomass, which likely explains that litter mass was reduced by 86.6% in burned plots (Table S7). In the unburned plots, litter mass was probably not only comprised of dead biomass produced in the 9 month period since fire but also from a longer time frame. Therefore, it is likely that the decrease in microbial C degradation genes in burned plots was driven by a strong decrease in both fresh and old aboveground plant material inputs to soil. Consistently, there was significant decrease in the relative abundance of the *amyX* and *glucoamylase* genes associated with the degradation of labile C in burned plots. The reduced access to labile C by microbial communities could be aggravated by the formation of severely or partly charred plant necromass (i.e., charcoal) by fire, which effectively absorbs soil organic C, especially plant root exudates (MacKenzie & DeLuca, 2006). Charcoal has a polycyclic aromatic structure that makes it chemically and biologically stable (Hart et al., 2005). However, we also observed decreases in the abundances of genes related to the degradation of chemically recalcitrant C, that is, chitin and lignin (Figure 2). Several mechanisms could offer explanations. First, absorbed soil organic C can block charcoal pores, preventing degradation of the charcoal surface by microbial enzymes as well as desorption of charcoal components into the solution (Zimmerman, Gao, & Ahn, 2011). Consistently, grass charcoal showed a negative priming effect in soil with low organic matter, reducing C mineralization rates (Zimmerman et al., 2011). Second, charred C is more resistant to degradation owing to the formation of mineral-organic aggregates (Czimczik, Preston, Schmidt, & Schulze, 2003; Jastrow, Amonette, & Bailey, 2006). Third, charcoal contains many toxic substances, such as dioxins, furans, phenols, and ethylene that reduce microbial activities, allowing charred C to reside in soil for a long time (Zimmerman et al., 2011).

However, we observed an increase in soil CO_2 efflux by 37% (Table S7), which was similar to the observation after the 2003 fire at the JRGCE during the second and third growing seasons postfire (Niboyet et al., 2011). Postfire increases in soil CO_2 efflux ranging from 10% to 33% were observed in many grassland systems (Knapp, Conard, & Blair, 1998; Munoz-Rojas, Lewandrowski, Erickson, Dixon, & Merritt, 2016; Xu & Wan, 2008). In our grassland, this effect was attributed to increased plant root biomass (Figure 4b), consistent with previous studies indicating that plants mediated the increased CO_2 efflux postfire in grassland ecosystems (Strong et al., 2017; Xu & Wan, 2008). This could be attributed to reduced early season shading by litter removal, and to a fertilization effect as litter combustion increased N and P inputs to soil (Henry, Chiariello, Vitousek, Mooney, & Field, 2006). Increased respiration rates from the increased root biomass and higher soil temperatures in the burned plots could also contribute to this plant-mediated mechanism (Strong et al., 2017). Alternatively, increased CO_2 efflux can be attributed to increased soil moisture (Knapp et al., 1998; Munoz-Rojas et al., 2016; Xu & Wan, 2008), though it could be more important in the short than medium and long terms. In contrast, when soil moisture was limited during the warm growing season, Vargas et al. (2012) also found that soil CO_2 efflux rates in an arid grasslands decreased by nearly 70% in 2-year postfire.

Bacterial community composition, as represented by the first principal component of bacterial community, was not significantly correlated with changes in soil respiration in burned sites (Figure 4b), reflecting a decreased microbial C degradation potential (Figure 2). In contrast, soil bacterial community composition was significantly correlated with plant aboveground biomass in burned sites (Figure 4b). Similarly, a previous study found that plant aboveground biomass and the flux of C assimilated to roots drove both microbial community composition and soil respiration (Litton, Ryan, Knight, & Stahl, 2003). The apparent lack of stimulated degradation of older soil C by microbial community, as shown by decreased soil C-degradation potential (Figure 2), suggests that there is a negative feedback to stabilize recalcitrant C post fire.

Fire is a strong controller of soil mineral N availability and plant community in semiarid grassland ecosystems. At the JRGCE, the 2003 fire increased soil NH_4^+ concentration (Docherty et al., 2011) but decreased the N:P ratio of plant tissue (Henry et al., 2006) in the first growing season postfire. At the second year after the 2003 fire, there was a significant decrease in soil NH_4^+ concentration, as well as a decreasing trend of gross N-mineralization rates (Niboyet et al., 2011). We found that the 2011 fire significantly decreased both soil NH_4^+ and NO_3^- concentrations (Table S7). Due to the low microbial activity and removal of aboveground plants, leaching likely occurred during the rainfalls post fire, leading to a substantial loss of inorganic N. Turner, Blair, Schartz, and Neel (1997) showed that fire reduced soil net N-mineralization rates. In contrast, total plant production 9 months after the 2003 fire also increased in the burned plots supplied with nitrate (Henry et al., 2006), while 33 months after the 2003 fire, aboveground and belowground plant biomasses were indistinguishable between the unburned and burned plots (Docherty et al., 2011). Both aboveground and belowground plant biomasses were increased by fire (Table S7). Therefore, the stimulatory effects of fire on plant biomass, possibly through the elimination of detrital shading and increase in soil temperature (Table S7), can offset the negative effects derived from reduced N supply. Our results suggest that postfire controls of soil N cycling and plant biomass may operate independently, leading to a large decoupling between an increased plant growth and decreased N cycling and availability in burned grasslands (Reich et al., 2001; Turner et al., 1997).

Many microbial genes related to N cycling were decreased in response to fire, which was likely due to the substantial decrease in litter biomass (from 166 to 22 g/m^2) and might also explain the reduced soil net N-mineralization rate often observed postfire (Reich et al., 2001; Turner et al., 1997). Consistent with decreased soil mineralization rates in burned northern forest ecosystems (Smithwick, Turner, Mack, & Chapin, 2005), we found that *ureC* —which is related to ammonification—was decreased (Figure 3). Although the pyrolysis of organic matter and the deposition of N in ash may have led to a short-lived pulse of soil NH_4^+ post fire (Smithwick et al., 2005), microbial genes related to N cycling tended to decrease in the longer term. For example, the relative abundances of genes associated with denitrification (e.g., *nirS* and *nosZ*) and dissimilatory nitrite reductase decreased steadily throughout several years postfire (Cobo-Diaz et al., 2015; Liu et al., 2013), which was attributed to reduced soil moisture and the availability of C and N substrates (Liu et al., 2013; Smithwick et al., 2005). However, almost 3 years after the 2003 fire disturbance, soil N_2O efflux and denitrifying enzyme activity remained higher in our burned plots (Niboyet et al., 2011). This inconsistency might be caused by different sampling time post fire disturbance.

Although fire did not change the abundance of *amoA* gene associated with nitrification, the abundance of *hao* gene—also associated with nitrification—was significantly decreased by 7.2% (Figure 3), which was concomitant with lower soil NH_4^+ concentration (Table S7). Several mechanisms might contribute to those results. First, increased soil temperatures could stimulate NH_4^+ to volatilize into atmosphere (Romanyà, Casals, & Vallejo, 2001). Indeed, fire caused a loss of 10–40 kg N ha^{-1} year^{-1} across a forest–grassland continuum by volatilization (Reich et al., 2001). Second, nitrification may be inhibited by charcoal (Turner, Smithwick, Metzger, Tinker, & Romme, 2007). Third, soil P could affect the competition between nitrifiers and other soil organisms. Although we did not measure the concentration of available P in soil, the significant decrease in P-cycling genes in relative abundance (Figure S5) suggested a decreased microbial potential for P cycling. Fourth, there might be an increased competition for soil N between plants and microbes, including nitrifiers, thus leading to lower nitrification rates (Figures 3 and 4).

Our findings highlight how fire affects microbial communities and soil biogeochemical cycling potential 9 months postfire, reflecting the recovery of the ecosystem during the first growing season post fire. First, our results add to growing evidence from multiple grassland ecosystems that fire increases growing season soil CO_2 efflux, suggesting that altered fire regimes could affect grassland C-turnover rates and increase greenhouse gas emissions from grasslands to atmosphere. As the grasslands in our study are used to frequent fires (Docherty et al., 2011), the plant community is likely to be highly resilient to infrequent, high-intensity fires by developing adaptive plant traits to fire regimes (Keeley & Brennan, 2012; Keeley, Pausas, Rundel, Bond, & Bradstock, 2011). This might contribute to higher postfire soil CO_2 efflux than that in grasslands that are not well adapted to frequent fire regimes (Knapp et al., 1998). Second, we demonstrate that negative biotic interactions and deterministic filtering have a key role regarding fire effects on the taxonomic composition of the bacterial community. Third, our study shows that the influence of fire on soil microorganisms and plants induces a decoupling between increased plant growth both above- and belowground, and decreased soil N-cycling potential and availability. Therefore, fire might exacerbate the competition for soil N between plants and soil microorganisms, then altering the competitive balance between them. Fire-induced N limitation in this grassland could also constrain the responses of plant and soil microorganisms to other global change factors, such as elevated CO_2, warming, and precipitation.

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