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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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46

47 **Running title:** Microbial responses to fire

48 **Key words:** Fire; Microbial communities; Climate change; Californian grasslands;

49 high-throughput sequencing; GeoChip

50 **Type of paper:** Primary research article

51 **Abstract**

52 Fire is a crucial event regulating the structure and functioning of many ecosystems.

53 Yet few studies have focused on how fire affects taxonomic and functional diversity of
54 soil microbial communities, along with changes in plant communities and soil carbon

55 (C) and nitrogen (N) dynamics. Here, we analyze these effects in a grassland

56 ecosystem nine-months after an experimental fire at the Jasper Ridge Global Change

57 Experiment (JRGCE) site in California, USA. Fire altered soil microbial communities

58 considerably, with community assembly process analysis showing that environmental
59 selection pressure was higher in burned sites. However, a small subset of highly
60 connected taxa were able to withstand the disturbance. In addition, fire decreased the
61 relative abundances of most functional genes associated with C degradation and N
62 cycling, implicating a slow-down of microbial processes linked to soil C and N
63 dynamics. In contrast, fire stimulated above- and below-ground plant growth, likely
64 enhancing plant-microbe competition for soil inorganic N, which was reduced by a
65 factor of about 2. To synthesize those findings, we performed structural equation
66 modeling, which showed that plants but not microbial communities were responsible
67 for significantly higher soil respiration rates in burned sites. Together, our results
68 demonstrate that fire “reboots” the grassland ecosystem by differentially regulating
69 plant and soil microbial communities, leading to significant changes in soil C and N
70 dynamics.

71 **Introduction**

72 Anthropogenic activities, and in particular changes in land use and management,
73 have considerably altered fire regimes (Andersson, Michelsen, Jensen, & Kjølner,
74 2004). Climate change is likely to also affect fire regimes because antecedent wet
75 periods could provide herbaceous fuels for fire, while drought and warming could
76 extend weather patterns that are conducive to fire (Bowman et al., 2009). As a
77 consequence, recent years have witnessed increased fire frequencies in many areas,
78 and it is anticipated that fire frequency and intensity will further increase in the coming
79 century (Westerling, Hidalgo, Cayan, & Swetnam, 2006). It is likely that fire in
80 California would burn more rapidly, with higher intensity, under warmer climate
81 scenarios (Fried, Torn, & Mills, 2004).

82 Fire affects plant diversity by increasing resource availability for subordinate and
83 early-successional species (Pickett, White, & Brokaw, 1985). Fire also has a
84 short-term stimulatory effect on N availability to plants but a long-term negative effect
85 due to increased N losses (Reich, Peterson, Wedin, & Wrage, 2001). Fire can
86 stimulate fine root production and aboveground net primary production in the first
87 post-fire growing season in grasslands (Docherty, Balser, Bohannon, & Gutknecht,
88 2011; Strong, Johnson, Chiariello, & Field, 2017). Changes in aboveground
89 communities could indirectly affect belowground systems via altering nutrient inputs,
90 increasing solar warming and surface soil temperatures, and changing
91 evapotranspiration and soil moisture (Hart, DeLuca, Newman, MacKenzie, & Boyle,

92 2005; Strong et al., 2017). Fire can also induce higher greenhouse gases emissions,
93 including CO₂ (Strong et al., 2017) and N₂O (Niboyet et al., 2011). Responses of soil
94 C dynamics to fire vary between ecosystems. For instance, soil respiration was
95 stimulated shortly after fire and then decreased in a tropical savanna woodland
96 (Andersson et al., 2004) and a permafrost forest (Tas et al., 2014), but CO₂ efflux
97 rates decreased after fire in a ponderosa pine forest (Sullivan et al., 2010) and an arid
98 grassland (Vargas et al., 2012). In a grassland ecosystem, three mechanisms –
99 increases in soil temperature, fine root production and aboveground net primary
100 production – were shown to explain changes in soil respiration rates post fire (Strong
101 et al., 2017).

102 Fire plays an important role in structuring and altering underground microbial
103 communities. Fire can directly kill microbes by heating the soil upper layer (Delmas et
104 al. 1995) and indirectly affect microbial community composition by altering plant
105 community composition and plant-driven organic matter inputs to soil (Docherty et al.,
106 2011; Hart et al., 2005), as well as soil environmental conditions. In boreal forests, fire
107 decreased microbial biomass and dissolved organic C in surface organic horizons
108 five years post fire (Waldrop & Harden, 2008). In contrast, fire stimulated microbial
109 growth by increasing soil ammonium (NH₄⁺) and dissolved organic C concentrations
110 by 20 - 52% in a tropical savannah woodland (Andersson et al., 2004). However,
111 microbial N immobilization and plant N uptake were reduced by fire. In grasslands,
112 enzyme activities related to C degradation and P cycling decreased, the effect being
113 mild in the first growing season after fire, higher in the second growing season after
114 fire, but subsidizing in the third growing season after fire (Gutknecht, Henry, & Balsler,
115 2010). In contrast, microbial enzyme activities related to organic N degradation have
116 been shown to either decrease (Gutknecht et al., 2010) or increase (Ajwa, Dell, &
117 Rice, 1999), after fire, likely due to site specific differences. As fire could strongly
118 affect soil nutrients, responses of microbial functional groups to pulse increases of
119 nutrient availability could cause long-term changes in ecosystem. For example, fire
120 significantly decreased soil microbial biomass and changed compositions of
121 nitrogen-fixing and ammonia-oxidizing communities in a mixed conifer forest, linking
122 to fire-related increases in soil NH₄⁺ or soil pH (Yeager, Northup, Grow, Barns, &
123 Kuske, 2005). This can change nitrification rates, NO and N₂O flux in post-fire
124 ecosystems (Serça et al., 1998). Overall, some inconsistencies among fire effects on
125 soil N processes have been reported, which reflects differences in soil type, fire

126 intensity, vegetation type and the timing of measurements after fire.

127 Microbe-mediated soil bioprocesses are considered to be one of the important
128 drivers of ecosystem recovery from fire (Neary, Klopatek, DeBano, & Ffolliott, 1999).
129 Although many studies have investigated the responses of below- and above-ground
130 communities to fire in grasslands, studies examining the fire effects on soil microbes
131 have largely focused on microbial population sizes and microbial activity (Ajwa et al.,
132 1999; Andersson et al., 2004; Gutknecht et al., 2010; Waldrop & Harden, 2008;
133 Yeager et al., 2005). In contrast, how fire affects the total soil microbial community
134 composition largely remains to be studied in grassland ecosystems, and it is unclear
135 which assembly processes control post-fire microbial community dynamics. Moreover,
136 it is needed to analyze fire effects not only on the taxonomic diversity of soil microbial
137 community, but also on its functional diversity, particularly the range of microbial
138 functional groups linked to soil C and N dynamics, is required to expand our
139 understanding of the effects of fire on soil function in grassland ecosystems.

140 Here, we studied fire effects in an annual Mediterranean grassland in California,
141 USA, as part of the long-term Jasper Ridge Global Change Experiment (JRGCE)
142 (Henry, Juarez, Field, & Vitousek, 2005). In particular, we examined fire effects on the
143 soil microbial community nine months after the prescribed burn in July 2011. We used
144 bacterial *16S rRNA* gene amplicon sequencing (Illumina MiSeq) and functional gene
145 array (GeoChip 4.6) analyses to examine the effects of fire on the taxonomic and
146 functional diversities of soil microbial communities. These techniques allow for
147 high-resolution profiling of community composition for both facets of microbial
148 diversity. We also examined whether the fire would have had cascading effects on
149 ecosystem functioning such as soil CO₂ efflux.

150 Given that fire typically increases soil C and N inputs, we hypothesized that there
151 would be a shift of microbial taxonomic composition, and more particularly that
152 functional genes associated with labile C degradation would be stimulated, leading to
153 higher soil CO₂ efflux. Since previous results at our study site showed that fire
154 significantly increased soil NH₄⁺ concentrations (Docherty et al., 2011) in the first
155 growing season post 2003 fire, and soil N₂O emission (Niboyet et al., 2011) in the
156 second and third growing seasons after a 2003 fire, we also hypothesized that
157 functional microbial groups associated with N cycling would be stimulated.

158

159 **Materials and methods**

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160 *Site description*

161 The Californian annual grassland ecosystem experiences a Mediterranean-type
162 climate, in which the growing season is cool and wet from November to May, and the
163 rest of the year is hot and dry. The plant community comprises non-native annual
164 grasses, perennial native grasses, native annual forbs and non-native forbs (Henry et
165 al., 2005). Soil mainly consists of weathered alluvium, which is a fine and mixed Typic
166 Haploxeralf. This experiment was initiated in October 1998 at the Jasper Ridge
167 Global Change Experiment (JRGCE) site located in central California, USA (37°24'N,
168 122°13'W). Initially, the JRGCE was designed to assess grassland ecosystem
169 responses to single and multiple components of global changes, including elevated
170 CO₂, warming, N deposition, and enhanced precipitation. To this end, each 2
171 m-diameter circular plot at the JRGCE was equally divided into four quadrants (i.e.,
172 subplots) of 0.78 m². N deposition and enhanced precipitation treatments were
173 arranged at the quadrant level, and the elevated CO₂ and warming treatments were
174 arranged at the plot level in a full factorial design. In the summer of 2003, an
175 accidental wildfire burned two of the eight blocks of the experiment (i.e. 32 of the 128
176 subplots). In the summer of 2011, a controlled fire was applied to four of the eight
177 blocks (i.e. 64 of the 128 subplots including the subplots that were already burned in
178 2003). Soil samples were collected on 26 and 27 April 2012, i.e. 9 months post fire
179 and 14 years after the experiment began. We collected one soil core with 5 cm
180 diameter x 7 cm deep per quadrant to generate each of 64 unburned and 64 burned
181 samples. We thoroughly mixed each sample and sieved soil through a 2 mm mesh.
182 Those soil samples were stored at -80°C prior to DNA extraction or at -20°C prior to
183 soil geochemical measurements.

184
185 *DNA extraction, purification and quantification*

186 DNA was extracted from 5 g of soil samples with a freeze-grinding mechanical lysis
187 method (Zhou, Bruns, & Tiedje, 1996). DNA was purified by gel electrophoresis with a
188 0.5% low-melting-point agarose, then extracted with phenol and chloroform and
189 finally precipitated with butanol. DNA quality was assessed by ratios of light
190 absorbance at A₂₆₀/A₂₈₀ (>1.8) and A₂₆₀/A₂₃₀ (>1.7), using a Nanodrop (NanoDrop
191 Technologies Inv., Wilmington, DE, USA). The final DNA concentration was
192 quantified using a PicoGreen® method using a FLUOstar Optima microplate reader
193 (BMG Labtech, Jena, Germany) (S. Liu et al., 2015).

194

195 *16S rRNA amplicon sequencing and raw data processing*

196 A PCR amplification targeting the V4 hypervariable region of 16S rRNA genes was
197 carried out to examine the bacterial community diversity, using the primers F515
198 (5'GTGCCAGCMGCCGCGG3') and R806 (3'TAATCTWTGGGVHCCATCAG5'). The
199 PCR products were sequenced by 2x250 bp paired-end sequencing with MiSeq
200 instrument (Illumina, San Diego, CA, USA). Raw sequence data were processed on
201 the Galaxy platform (<http://zhoulab5.rccc.ou.edu:8080>). The paired-end raw
202 sequences were demultiplexed to remove PhiX sequences and then sorted to
203 appropriate samples based on paired barcodes. These sequences were then
204 trimmed with Btrim based on quality scores and paired-end reads were combined
205 with FLASH into full-length sequences (Magoc & Salzberg, 2011). Sequences less
206 than 200 bp or containing ambiguous bases were removed and chimeric sequences
207 were discarded based on prediction by Uchime using the reference database mode.
208 Operational taxonomic units (OTUs) were clustered at the 0.97 identity level by
209 UCLUST (R.C. Edgar, 2010; R. C. Edgar, Haas, Clemente, Quince, & Knight, 2011).
210 Taxonomic annotation was assigned to OTUs based on representative sequences
211 with RDP's 16S Classifier. The number of sequences retrieved from each sample
212 might differ, and this reflects different efficiency of the sequencing process rather than
213 actual biological variation (Weiss et al., 2017). We thus standardized the number of
214 sequences across samples.

215

216 *GeoChip hybridization and raw data processing*

217 To study functional genes of bacterial, archaeal and fungal communities, DNA
218 hybridization with GeoChip 4.6 was carried out as previously described (Y. Yang et al.,
219 2013). Microbial DNA extracted from soil was labeled with Cy-3 fluorescent dye using
220 random priming method, and then purified with a DNA purification kit (Qiagen,
221 Valencia, CA, USA). After drying at 45°C with SpeedVac (ThermoSavant, Milford, MA,
222 USA), labeled DNA was hybridized to GeoChip 4.6 at 42°C for 16 h in a MAUI
223 hybridization system (BioMicro, Salt Lake City, UT, USA). After washing the slides
224 with buffers provided by NimbleGen, the hybridized DNA was scanned using a
225 NimbleGen MS 200 Microarray Scanner (Roche, Basel, Switzerland).

226 Raw GeoChip data were processed as follows: (i) remove the spots with a
227 signal-to-noise ratio (SNR) less than 2.0; (ii) perform log-transformation on the signal

228 intensity for each gene and divide them by the mean intensity in the sample; and (iii)
229 remove genes detected only in one out of the four replicates from the same
230 treatment.

231

232 *Soil abiotic measurements*

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

247

248 *Vegetation variable measurements*

249 Aboveground plant biomass was collected from 141 cm^2 of each quadrant on the
250 sampling in April 2012. Individual plant species were separated into functional groups
251 (annual grasses, perennial grasses, annual forbs, perennial forbs, and litter), and the
252 biomass was oven-dried at 70°C and weighed. Root biomass was estimated by
253 separating roots from two soil cores (2.5 cm diameter by 15 cm depth) from the same
254 area of the aboveground biomass collection, and the root biomass was oven-dried at
255 70°C and weighed.

256

257 *Statistical analyses*

258 Unpaired *t*-tests were used to determine fire effects on relative abundances of
259 microbial taxa, functional genes and soil and vegetation variables. *P* values were
260 adjusted by the Benjamini-Hochberg method with sequentially modified Bonferroni
261 Correction, using the `p.adjust` function in the `vegan` package in R version 3.5.0.

262 Permutational multivariate ANOVA (PERMANOVA) was implemented to rank global
263 change drivers of the composition of environmental variables, using adonis function in
264 the vegan package. Analysis of variance (ANOVA) was performed to rank global
265 change drivers of each environmental variable, using aov function in the vegan
266 package. Three nonparametric dissimilarity tests (Multi-response permutation
267 procedure (mrpp), Analysis of similarities (anosim) and Permutational multivariate
268 analysis of Variance (adonis) in the vegan package in R) were used to compare
269 differences of microbial community composition. Shannon Index (H') was used to
270 calculate the α -diversity of the microbial community in the unburned and burned plots,
271 using the alpha.g function in the ieggr package in R.

272 Microbial community assembly could be shaped by interspecies competition for
273 resources and cooperative interactions (Zelezniak et al., 2015). Metabolically
274 interdependent microbial groups are strongly coupled through mutualistic interactions
275 which support species growth: thus these groups form persistent sub-networks within
276 the soil microbial communities (Zelezniak et al., 2015). Such high degree of
277 connectivity among keystone taxa can dampen soil microbial compositional changes
278 in response to environmental changes and disturbances (Herren & McMahon, 2018;
279 Zelezniak et al., 2015). Therefore, we used linear mixed model to examine whether
280 the connectivity among keystone taxa, measured by cohesion metrics, could explain
281 microbial compositional changes. Cohesion metrics measure the instantaneous
282 connectivity of a microbial community and contain two metrics showing the strength
283 of positive and negative relationships between taxa. Bray-Curtis dissimilarity was
284 used to measure community compositional changes at OTUs level. The regression
285 analysis using the most highly connected taxa was conducted to examine the
286 relationship between Bray-Curtis dissimilarity and cohesion metrics. The R^2 of each
287 model was recorded. Highly connected taxa would be better predictors of the whole
288 bacterial community than randomly selected taxa since the former has
289 disproportionate influence in determining bacterial community dynamics. Regression
290 analyses using random subsets of taxa were repeated 500 times to generate a
291 distribution of model R^2 . We compared the R^2 value of a model using the most highly
292 connected taxa with that of models using random taxa: if the former is higher than the
293 95th percentile of the latter, then the model using the most highly connected taxa was
294 considered to be significantly different from the model using random taxa, suggesting
295 that interactions among keystone taxa indeed have an important role regarding

296 compositional changes of the bacterial community. In addition, we used the `pnull`
297 function in the `iegr` package in R to calculate the β -nearest taxon index (β NTI),
298 which measures the degree to which microbial community composition is governed
299 by environmental filtering based on OTUs' ecological niches (Stegen et al., 2013).

300 Using the `psem` function in the `piecewiseSEM` package in R, structural equation
301 models (SEM) were also fitted to calculate the direct and indirect effects of soil
302 variables, soil microbial community composition and plant biomass on soil CO₂ efflux
303 rates. Model fits were measured by a non-significant Shipley's test of d-separation,
304 with Fisher's $P > 0.05$ indicating that no paths were missing, and low Akaike
305 information criterion (AIC) being used to choose among competing models (Jing et al.,
306 2015). The first principal components (PCo1) of microbial taxonomic community were
307 used to represent the microbial community composition, using the `pco` function in the
308 `vegan` package in R.

309

310 **Results**

311 The fire impact on the soil bacterial community did not vary largely across different
312 levels of CO₂, nitrogen deposition, warming and precipitation (Table S1). Furthermore,
313 of over 26 possible interactive effects tested between fire and other global climate
314 changes, only one was statistically significant - the interaction between fire and
315 nitrogen deposition on soil nitrate concentration (Table S2 & S3). Since the
316 responses to fire across the sixteen factorial combinations of CO₂, nitrogen
317 deposition, warming and precipitation (n=64) were similar to those for fire as a sole
318 treatment (n=4) (Table S1), data were pooled across all the other treatment levels
319 following a previous example (Reich et al., 2006). The n=64 samples increased the
320 statistical power and identified trends valid across the range of climate change
321 scenarios tested at the Jasper Ridge Global Change Experiment.

322

323 *Changes of taxonomic composition of bacterial communities by fire*

324 A total of 8,882,825 sequences were obtained, ranging from 29,275 to 92,183 reads
325 per sample. After re-sampling with 29,275 sequences per sample, 87,275 operational
326 taxonomic units (OTUs) were obtained. Singletons were removed, which might
327 represent inherently unreplicated data possibly resulting from sequencing errors
328 (Dickie, 2010), leading to a total of 64,515 OTUs. Using three non-parametric
329 statistical analyses (Anosim, Adonis, and MRPP), we found that fire significantly

330 changed taxonomic composition ($P \leq 0.003$ for all three methods, Table 1). However,
331 the α -diversity of the bacterial community, based on Shannon index, was similar
332 between the unburned (8.16 ± 0.02) and burned (8.24 ± 0.02) sites ($P = 0.270$). 4,503
333 unique OTUs were detected in unburned plots, and 6,744 unique OTUs in burned
334 plots, while 53,268 OTUs were found in both plots (Fig. S1a). The relative abundance
335 of *Proteobacteria* was significantly higher in burned plots ($P = 0.047$), while the
336 relative abundances of *Planctomycetes* ($P = 0.047$), *OD1* ($P = 0.009$), *Chlorobi* ($P =$
337 0.009), *OP11* ($P = 0.007$) and *Spirochaetes* ($P = 0.006$) were significantly lower in
338 burned plots (Fig. S1b). At finer taxonomic levels, relative abundances of 19 genera
339 (such as *Agromyces*, *Ferruginibacter* and *Pasteuria*) were significantly decreased,
340 whereas those of 16 genera (such as *Actinoplanes*, *Bdellovibrio* and *Flavisolibacter*)
341 were significantly increased (Table S4).

342 Cohesion metrics were significant predictors of compositional changes in burned
343 sites ($P = 0.017$ and $P < 0.001$) (Table S5), with both the negative and positive
344 cohesion metrics related to the compositional changes ($P < 0.001$) (Fig. S2). The R^2
345 values of models using the most highly connected taxa remained unchanged when
346 the least-connected taxa were removed, whereas the R^2 values of models containing
347 random subsets of taxa decreased when more taxa were removed (Fig. 1a). The
348 explanatory power of these models increased to 13% when a small subset (46) of
349 highly connected taxa was used to calculate the cohesion metrics, suggesting that
350 biotic interactions among taxa could partially explain the community compositional
351 change. These keystone taxa included 13 genera from *Proteobacteria* (such as
352 *Rudaea*, *Bauldia* and *Microvirga*), 9 genera from *Acidobacteria* (*Gp1-Gp7*, *Gp16* and
353 *Gp17*), 6 genera from *Actinobacteria* (such as *Rugosimonospora*, *Catenulispora* and
354 *Agromyces*), 6 genera from *Bacteroidetes* (such as *Terrimonas*, *Adhaeribacter* and
355 *Ferruginibacter*), 4 genera from *Planctomycetes* (*Gemmata*, *Planctomyces*,
356 *Singulisphaera* and *Isosphaera*), and 7 genera from *Armatimonadetes*, *Chloroflexi*,
357 *Firmicutes*, *Verrucomicrobia* and *WS3* (Table S6).

358 In burned sites, the models with highly connected taxa showed significantly
359 higher performance than those with randomly selected taxa (Fig. 1a). As the negative
360 cohesion was a better predictor of compositional changes of the bacterial community
361 than positive cohesion (Fig. S2), we focused on 46 genera showing the strongest
362 negative associations with others. Among them, 7 genera of *Gp5* ($P = 0.039$),
363 *Agromyces* ($P = 0.010$), *Ferruginibacter* ($P = 0.039$), *Pasteuria* ($P = 0.001$),

364 *Planctomyces* ($P = 0.001$), *Isosphaera* ($P = 0.041$) and *Verrucomicrobium* ($P < 0.001$)
365 decreased in relative abundances compared to control (Fig. S3). Many of those highly
366 connected genera had low relative abundances. For example, *Rudaea* was the 9th
367 most connected genus but its relative abundance ranked only the 144th among all the
368 genera, with a mean relative abundance across all treatments of 0.03% (Table S6). In
369 unburned sites, the explanatory power of models constructed using either randomly
370 selected or most connected taxa remained very low ($< 2\%$, Fig. 1a), suggesting that
371 there was no keystone taxa effect in the unburned sites.

372 The β -nearest taxon index (β NTI), indicating the degree to which bacterial
373 community composition is governed by environmental filtering, increased from $3.28 \pm$
374 0.025 in unburned plots to 3.74 ± 0.027 in burned plots ($P < 0.001$) (Fig. 1b),
375 suggesting that the bacterial community was more strongly governed by
376 environmental filtering in burned plots.

377

378 *Changes of microbial functional potentials by fire*

379 A total of 68,695 microbial functional genes were detected by GeoChip. Fire caused
380 significant changes in the overall functional composition of soil microbial communities
381 according to the three non-parametric statistical analyses conducted ($P < 0.001$)
382 (Table 1) and significantly decreased microbial functional α -diversity.

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

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

395

396 *Fire influence on soil and vegetation variables*

397 After nine months post fire, aboveground plant biomass (+25.8%, $P = 0.034$) and

398 belowground biomass increased (+24.6%, $P = 0.080$) but litter mass decreased
399 (-86.6%, $P < 0.001$) (Table S7). Soil temperature also increased (+1°C, $P < 0.001$)
400 (Table S7). Soil NO_3^- decreased from 346 mg L⁻¹ to 189 mg L⁻¹ ($P = 0.029$), and soil
401 NH_4^+ from 834 mg L⁻¹ to 389 mg L⁻¹ ($P = 0.034$) (Table S7). Soil CO_2 efflux
402 significantly increased in the burned plots (+ 37.1%, $P < 0.001$) (Table S7). Other soil
403 variables such as soil moisture, pH and TN remained unchanged by fire (Table S7).

404

405 *Drivers of changes in soil functioning and bacterial communities*

406 In the unburned sites, soil CO_2 efflux was correlated to root biomass ($\beta = 0.436$, $P =$
407 0.003) and to a similar extent to bacterial community composition ($\beta = 0.421$, $P =$
408 0.006) (Fig. 4a). In contrast, soil CO_2 efflux in burned sites was only and strongly
409 correlated to root biomass ($\beta = 0.936$, $P = 0.044$, Fig. 4b). In these burned sites,
410 bacterial community composition was related to aboveground plant biomass ($\beta =$
411 0.205, $P = 0.011$) and soil moisture ($\beta = 0.171$, $P = 0.043$), though soil pH ($\beta = 0.610$,
412 $P < 0.001$) had stronger effects on microbial community (Fig. 4b).

413

414 **Discussion**

415 Fire significantly altered the taxonomic composition of the bacterial community. The
416 change could be predicted by cohesion metrics revealing the existence of 'keystone'
417 taxa, which exert disproportionate influence on community composition through
418 strong biotic interactions (Herren & McMahon, 2018). Biotic interactions can include
419 metabolite exchanges among taxa, which enhances self-reinforcing modules within
420 microbial communities that can stabilize community composition (Levy & Borenstein,
421 2013). More particularly, stronger negative cohesion was related to lower levels of
422 compositional change in the burned sites (Table S5). Those strong negative cohesion
423 values represented the negative feedback loops formed by biotic interactions among
424 taxa, which had an important role in stabilizing community composition and mitigating
425 the effects of fire. Similar results were reported for other environmental disturbances
426 (Coyte, Schluter, & Foster, 2015; Konopka, Lindemann, & Fredrickson, 2015). This is
427 also consistent with recent studies reporting that negative interactions among taxa,
428 such as competition and predation, prevail in microbial communities (Herren &
429 McMahon, 2018). For instance, a previous study focusing on bacterial strains isolated
430 from a rainwater pool showed that negative interactions accounted for a great
431 majority of pairwise taxa interactions, owing to potential resource competition among

432 microbes (Foster & Bell, 2012). Since microbial diversity is typically high, investing in
433 positive interactions often specific to a few other species could bring little evolutionary
434 benefit, which may explain the dominance of negative interactions (Foster & Bell,
435 2012).

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

455 In sharp contrast to higher above- and belowground plant biomass in response to
456 fire (Table S7), most of the microbial genes related to C degradation were
457 significantly decreased (Fig. 2). Among all the 16,326 C degradation genes detected
458 in our study, 1.9% were archaeal genes, 14.7% were fungal genes and 83.3% were
459 bacterial genes. Archaeal, fungal and bacterial C degradation genes were affected by
460 fire to similar extent, approximately one-third of each category being significantly
461 changed. Fire largely consumed the standing dead plant biomass, which likely
462 explains that litter mass was reduced by 86.6% in burned plots (Table S7). In the
463 unburned plots, litter mass was probably not only comprised of dead biomass
464 produced in the nine months period since fire but also from a longer time frame.
465 Therefore, it is likely that the decrease in microbial C degradation genes in burned

466 plots was driven by a strong decrease in both fresh and old aboveground plant
467 material inputs to soil. Consistently, there was significant decrease in the relative
468 abundance of the *amyX* and *glucoamylase* genes associated with degradation of
469 labile C in burned plots. The reduced access to labile C by microbial communities
470 could be aggravated by the formation of severely or partly charred plant necromass
471 (i.e., charcoal) by fire, which effectively absorbs soil organic C, especially plant root
472 exudates (MacKenzie & DeLuca, 2006). Charcoal has indeed a polycyclic aromatic
473 structure that makes it chemically and biologically stable (Hart et al., 2005). However,
474 we also observed decreases in the abundances of genes related to the degradation
475 of chemically recalcitrant C, i.e., chitin and lignin (Fig. 2). Several mechanisms could
476 offer explanations. First, absorbed soil organic C can block charcoal pores,
477 preventing degradation of the charcoal surface by microbial enzymes as well as
478 desorption of charcoal components into the solution (Zimmerman, Gao, & Ahn, 2011).
479 Consistently, grass charcoal showed a negative priming effect in soil with low organic
480 matter, reducing C mineralization rates (Zimmerman et al., 2011). Second, charred C
481 is more resistant to degradation owing to the formation of mineral-organic aggregates
482 (Czimeczik, Preston, Schmidt, & Schulze, 2003; Jastrow, Amonette, & Bailey, 2006).
483 Third, charcoal contains many toxic substances, such as dioxins, furans, phenols and
484 ethylene, that reduce microbial activities, allowing charred C to reside in soil for a long
485 time (Zimmerman et al., 2011).

486 However, we observed an increase of soil CO₂ efflux by 37% (Table S7), which
487 was similar to the observation after the 2003 fire at the JRGCE during the second and
488 third growing seasons post fire (Niboyet et al. 2011). Post-fire increases of soil CO₂
489 efflux ranging from 10% to 33% were observed in many grassland systems (Knapp ,
490 Conard, & Blair, 1998; Munoz-Rojas, Lewandrowski, Erickson, Dixon, & Merritt, 2016;
491 Xu & Wan, 2008). In our grassland, this effect was attributed to increased plant root
492 biomass (Fig. 4b), consistent with previous studies indicating that plants mediated the
493 increased CO₂ efflux post-fire in grassland ecosystems (Strong et al., 2017; Xu &
494 Wan, 2008). This could be attributed to reduced early season shading by litter
495 removal, and to a fertilization effect as litter combustion increased N and P inputs to
496 soil (Henry, Chiariello, Vitousek, Mooney, & Field, 2006). Increased respiration rates
497 from the increased root biomass and higher soil temperatures in the burned plots
498 could also contribute to this plant-mediated mechanism (Strong et al., 2017).
499 Alternatively, increased CO₂ efflux can be attributed to increased soil moisture

500 (Knapp et al., 1998; Munoz-Rojas et al., 2016; Xu & Wan, 2008), though it could be
501 more important in the short than medium and long terms. In contrast, when soil
502 moisture was limited during the warm growing season, Vargas et al. (2012) also
503 found that soil CO₂ efflux rates in an arid grasslands decreased by nearly 70% in
504 2-year post fire.

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

515 Fire is a strong controller of soil mineral N availability and plant community in
516 semi-arid grassland ecosystems. At the JRGCE, the 2003 fire increased soil NH₄⁺
517 concentration (Docherty et al., 2011) but decreased the N:P ratio of plant tissue
518 (Henry et al., 2006) in the first growing season post fire. At the second year after the
519 2003 fire, there was a significant decrease of soil NH₄⁺ concentration, as well as a
520 decreasing trend of gross N mineralization rates (Niboyet et al., 2011). We found that
521 the 2011 fire significantly decreased both soil NH₄⁺ and NO₃⁻ concentrations (Table
522 S7). Due to the low microbial activity and removal of aboveground plants, leaching
523 likely occurred during the rainfalls after fire, leading to a substantial loss of inorganic
524 N. In accordance, C. L. Turner, Blair, Scharz, and Neel (1997) showed that fire
525 reduced soil net N mineralization rates. In contrast, total plant production nine months
526 after the 2003 fire also increased in the burned plots supplied with nitrate (Henry et al.
527 2006), while 33 months after the 2003 fire, aboveground and belowground plant
528 biomasses were indistinguishable between the unburned and burned plots (Docherty
529 et al., 2011). Both aboveground and belowground plant biomasses were increased by
530 fire (Table S7). Therefore, the stimulatory effects of fire on plant biomass, possibly
531 through the elimination of detrital shading and increase of soil temperature (Table S7),
532 can offset the negative effects derived from reduced N supply. Our results suggest
533 that post-fire controls of soil N cycling and plant biomass may operate independently,

534 leading to a large decoupling between an increased plant growth and decreased N
535 cycling and availability in burned grasslands (Reich et al., 2001; C. L. Turner et al.,
536 1997).

537 Many microbial genes related to N cycling were decreased in response to fire,
538 which was likely due to the substantial decrease of litter biomass (from 166 to 22 g/m²)
539 and might also explain the reduced soil net N mineralization rate often observed post
540 fire (Reich et al., 2001; C. L. Turner et al., 1997). Consistent with decreased soil
541 mineralization rates in burned northern forest ecosystems (Smithwick, Turner, Mack,
542 & Chapin, 2005), we found that *ureC* –which is related to ammonification– was
543 decreased (Fig. 3). Although the pyrolysis of organic matter and the deposition of N in
544 ash may have led to a short-lived pulse of soil NH₄⁺ after fire (Smithwick et al., 2005),
545 microbial genes related to N cycling tended to decrease in the longer term. For
546 example, the relative abundances of genes associated with denitrification (e.g., *nirS*
547 and *nosZ*) and dissimilatory nitrite reductase decreased steadily throughout several
548 years post fire (Cobo-Diaz et al., 2015; X. Liu et al., 2013), which was attributed to
549 reduced soil moisture and availability of C and N substrates (X. Liu et al., 2013;
550 Smithwick et al., 2005). However, almost three years after the 2003 fire disturbance,
551 soil N₂O efflux and denitrifying enzyme activity remained higher in our burned plots
552 (Niboyet et al., 2011). This inconsistency might be caused by different sampling time
553 after fire disturbance.

554 Although fire did not change the abundance of *amoA* gene associated with
555 nitrification, the abundance of *hao* gene –also associated with nitrification– was
556 significantly decreased by 7.2% (Fig. 3), which was concomitant with lower soil NH₄⁺
557 concentration (Table S7). Several mechanisms might contribute to those results. First,
558 increased soil temperatures could stimulate NH₄⁺ to volatilize into atmosphere
559 (Romanyà, Casals, & Vallejo, 2001). Indeed, fire caused a loss of 10-40 kg N ha⁻¹ yr⁻¹
560 across a forest-grassland continuum by volatilization (Reich et al., 2001). Second,
561 nitrification may be inhibited by charcoal (M. G. Turner, Smithwick, Metzger, Tinker, &
562 Romme, 2007). Third, soil P could affect the competition between nitrifiers and other
563 soil organisms. Although we did not measure the concentration of available P in soil,
564 the significant decrease of P cycling genes in relative abundance (Fig. S5) suggested
565 a decreased microbial potential for P cycling. Fourth, there might be an increased
566 competition for soil N between plants and microbes, including nitrifiers, thus leading
567 to lower nitrification rates (Fig. 3 & 4).

568 Our findings highlight how fire affects microbial communities and soil
569 biogeochemical cycling potentials nine months post fire, reflecting the recovery of the
570 ecosystem during the first growing season after fire. First, our results add to growing
571 evidence from multiple grassland ecosystems that fire increases growing-season soil
572 CO₂ efflux, suggesting that altered fire regimes could affect grassland C turnover
573 rates and increase greenhouse gas emissions from grasslands to atmosphere. As the
574 grasslands in our study are used to frequent fires (Docherty et al., 2011), the plant
575 community is likely to be highly resilient to infrequent, high-intensity fires by
576 developing adaptive plant traits to fire regimes (Keeley & Brennan, 2012; Keeley,
577 Pausas, Rundel, Bond, & Bradstock, 2011). This might contribute to higher post-fire
578 soil CO₂ efflux than that in grasslands that are not well adapted to frequent fire
579 regimes (Knapp et al., 1998). Second, we demonstrate that negative biotic
580 interactions and deterministic filtering have a key role regarding fire effects on the
581 taxonomic composition of the bacterial community. Third, our study shows that fire
582 influence on soil microorganisms and plants induces a decoupling between increased
583 plant growth both above- and below-ground, and decreased soil N cycling potentials
584 and availability. Therefore, fire might exacerbate the competition for soil N between
585 plants and soil microorganisms, then altering the competitive balance between them.
586 Fire-induced N limitation in this grassland could also constrain the responses of plant
587 and soil microorganisms to other global change factors, such as elevated CO₂,
588 warming and precipitation.

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597

598 **Conflict of interest**

599 The authors declare no conflict of interest.

600

601 **Data availability**

602 GeoChip data are available online (www.ncbi.nlm.nih.gov/geo/) with the accession
603 number GSE107168 and GSE126737. MiSeq data are available in NCBI SRA
604 database with the accession number PRJNA419455.

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759 **Tables**

760 **Table 1.** Results of statistical analyses testing for a fire effect on the taxonomic and
761 functional compositions of microbial communities. Significant effects are indicated in
762 bold.

Statistical Approaches		Taxonomic	Functional
MRPP	Δ	0.479	0.287
	<i>P</i>	0.001	<0.001
Anosim	R	0.069	0.117
	<i>P</i>	0.001	0.001
Adonis	F	2.36	10.087
	<i>P</i>	0.003	<0.001

763

764 **Figure captions**

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

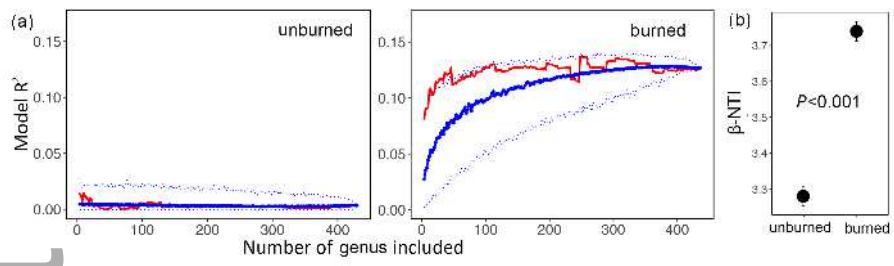
777
778 **Fig. 2:** Percentage changes in relative abundances of C degradation genes induced
779 by fire, calculated as: $100 * ((\text{mean value for burned samples} / \text{mean value for unburned}$
780 $\text{samples}) - 1)$. The complexity of C is arranged from labile (on the left) to recalcitrant
781 (on the right). Mean values and standard errors are presented ($n = 64$). Significant
782 negative changes in the relative abundances of the genes by fire are indicated in blue,
783 and significant positive changes in the relative abundances of the genes by fire are
784 indicated in red. Asterisks indicate significant differences: *, $P < 0.05$; **, $P < 0.01$; ***,
785 $P < 0.005$. P values were adjusted by the Benjamini-Hochberg method using
786 sequentially modified Bonferroni Correction.

787
788 **Fig. 3:** Percent changes in relative abundances of N cycling genes induced by fire,
789 calculated as: $100 * ((\text{mean value in burned samples} / \text{mean value in unburned samples})$
790 $- 1)$. Average signal intensities of the genes in blue were significantly decreased by
791 fire. Average signal intensities of the genes in black were not significantly altered by
792 fire. Genes in grey were not targeted by GeoChip. Asterisks indicate significant
793 differences: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. P values were adjusted by the
794 Benjamini-Hochberg method using sequentially modified Bonferroni Correction.

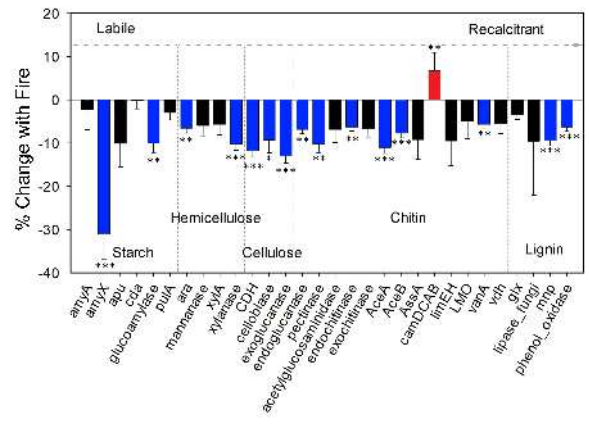
795
796 **Fig. 4:** Structural equation models using soil variables, bacterial community
797 composition and above- and below-ground plant biomass as predictors of soil CO_2

798 efflux rate for (a) unburned and (b) burned plots. Solid lines represent significant
799 paths ($P < 0.05$, piecewise SEM), and dashed lines represent insignificant paths ($P >$
800 0.05 , piecewise SEM). Blue arrows represent negative paths, and black arrows
801 represent positive paths. NO_3^- , soil nitrate concentration; bacterial community
802 composition: the first principal components (PCo1) of the principal co-ordinate
803 analysis of soil bacterial community. Overall fit of piecewise SEM was evaluated
804 using Shipley's test of d-separation, with Fisher's C statistics (if $P > 0.05$, then no
805 paths are missing, and the model is a good fit) and Akaike information criterion (AIC).
806 For each variable, the percentage of the variance explained by the model is indicated
807 next to the box corresponding to this variable.

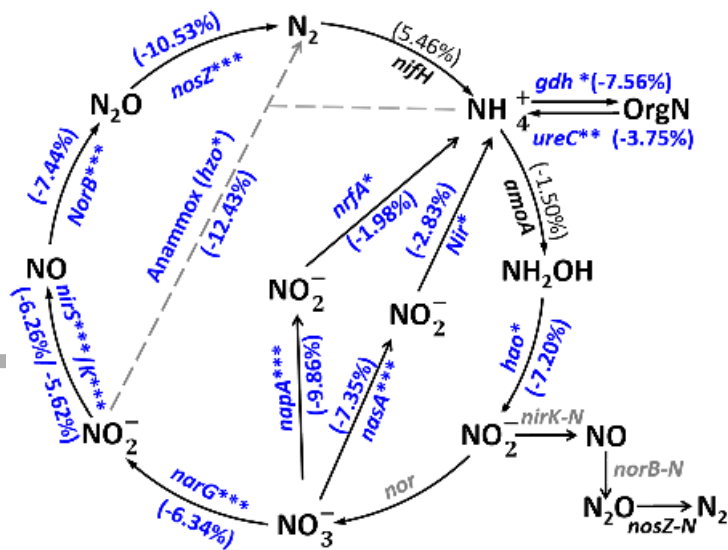
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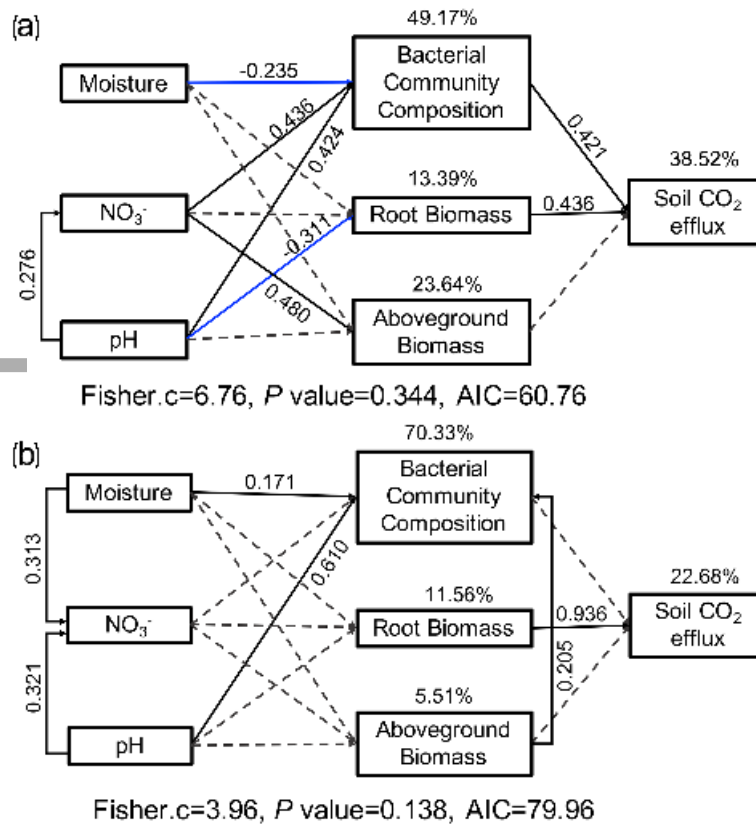
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