FEMS Microbiology Ecology Advance Access published December 5, 2014

1 **Climate-induced die-off affects plant-soil microbe ecological relationship and**
2 **functioning**

3 Francisco Lloret[1,2*,] Stefania Mattana[1], Jorge Curiel Yuste[1,3]

4

5 1. CREAF. E-08193 Cerdanyola del Vallès (Barcelona), Spain

6 2. U. Ecologia, Dept. Biologia Animal, Biologia Vegetal i Ecologia, Universitat
7 Autònoma Barcelona, E-08193 Cerdanyola del Vallès (Barcelona), Spain

8 3. Museo Nacional de Ciencias Naturales (MNCN), CSIC, Serrano 115 dpdo. E-28006
9 Madrid. Spain

10

11

12

13

14

15 * corresponding author, e-mail: francisco.lloret@uab.cat, Phone: +34935812700,
16 Address: CREAF, Edifici C, Campus Universitat Autònoma Barcelona, 08193
17 Cerdanyola del Vallès, Barcelona, Spain.

18

19

20

21

22

23

Key words

climate change, drought episode, extreme climatic event, microbial diversity, soil microbial community, microbial soil respiration

Abstract

This study reports the relationship between the diversity and functioning of fungal and bacterial soil communities with vegetation in Mediterranean woodland that experienced severe die-off after a drought episode. Terminal Restriction Fragment Length Polymorfism (TRFLP) was used to describe microbial community structure and diversity five years after the episode in different habitats (*Juniperus* woodland, shrubland, grassland), when the vegetation had not yet recovered.

Vegetation diversity was positively related to TRF bacterial richness under unaffected canopies and was higher in diverse grassland. Fungal TRF richness correlated with vegetation type, being greater in *Juniperus* woodland. Microbial respiration increased in grassland, whereas microbial biomass, estimated from soil substrate-induced respiration, decreased with bacterial diversity. Die-off increased bacterial richness and changed bacterial composition, particularly in *Juniperus* woodland, where herbaceous species increased, while fungal diversity was reduced in *Juniperus* woodland. Die-off increased microbial respiration rates.

The impact on vegetation from extreme weather episodes spread to microbial communities by modifying vegetation composition and litter quantity and quality, particularly as a result of the increase in herbaceous species. Our results suggest that climate-induced die-off triggers significant cascade effects on soil microbial communities, which may in turn further influence ecosystem C dynamics.

53 **1. Introduction**

54

55 The structure and functioning of soil microbial communities are sensitive to climate
56 change (e.g., Waldrop & Firestone, 2006; Sheik et al., 2011; Deslippe et al., 2012).
57 However, the response of these communities to extreme climate episodes remains largely
58 unknown (Berard et al., 2011; Meisner et al., 2013). In addition to the observed rise in
59 earth temperature, episodes of climatic anomaly, such as intense drought events, are
60 expected to become more frequent and intense in the coming decades in many regions
61 (IPCC, 2013). These extreme drought events may produce generalized vegetation die-off
62 and mortality (van Mantgem et al., 2009; Allen et al., 2010), particularly in the
63 Mediterraen Basin region (Lloret et al., 2004). The response of soil microbial
64 communities to extreme climatic events is to some extent determined by the impact on
65 standing vegetation, because vegetation alteration produces changes in soil microclimate,
66 soil organic matter (SOM), and rhizosphere (Bardgett et al., 2008; Sagova-Mareckova et
67 al., 2011). Therefore, understanding the impact of extreme weather events implies prior
68 knowledge of the relationship between soil microbial communities and standing
69 vegetation, followed by an appraisal of how this relationship is modified when a die-off
70 occurs.

71

72 Apart from soil chemistry, vegetation controls key processes that influence soil microbial
73 communities (Zak et al., 2003; Waldrop et al. 2006; Ushio et al., 2008; Mitchell et al.,
74 2012), particularly those determining litter quantity and quality (Bais et al., 2006).
75 Nevertheless, the relationship between the diversity of above-ground vegetation and soil
76 microbial communities has not been analyzed in many ecological contexts. Previous
77 studies have often merely described broad patterns at a regional scale or compared
78 contrasting habitats (but see Eskelinen et al., 2009; Cregger et al., 2012). This
79 relationship may be altered under strong abiotic stress, thereby causing significant
80 damage to vegetation.The resulting outcomes, however, are uncertain: soil microbial
81 diversity can be diminished because losses in vegetation diversity would reduce the
82 chemical diversity of the substrate; alternatively, vegetation die-off can increase the

83 amount of dead organic matter for microbial consumption, thus favoring microhabitat
84 heterogeneity.

85

86 This study described the diversity, structure, and functioning of soil microbial
87 communities in three types of vegetation (grassland, shrubland and *Juniperus* woodland)
88 of Mediterranean coastal South Spain that experienced a severe episode of drought and
89 cold temperature. This episode resulted in an extensive die-off of the woody vegetation
90 (shrubland and juniper-woodland) (del Cacho & Lloret, 2012). One characteristic of this
91 study system was the homogeneity of the soil substrate, which consists of recent sandy
92 deposits. This homogeneity minimized chemical and structural variability at the micro-
93 scale level. We used vegetation surveys to estimate plant diversity. Fingerprinting of
94 bacterial and fungal communities was obtained by examining terminal restriction
95 fragment length polymorphisms (T-RFLP) of PCR-amplified of bacterial 16S rDNA gene
96 and fungal ITS (Internal Transcribed Space) region from rDNA extracted from soil
97 samples. This molecular technique has been widely used to describe the structure and
98 diversity of these soil communities (e.g. Kennedy et al., 2005; CurielYuste et al., 2011).
99 Although the TRFLP technique cannot recognize taxonomic or functional groups, it does
100 facilitate satisfactory replication, thus permitting comparisons between communities'
101 diversity and structural patterns. We also explored variables that describe the functioning
102 of soil microbial communities (microbial respiration, microbial biomass, and metabolic
103 quotient) and other abiotic variables describing the physical-chemical conditions of the
104 uppermost part of the soil profile, such as pH and soil organic matter (SOM) content.

105 The specific objectives of the study were:

106 - To analyze the contribution of (i) vegetation type, and (ii) climate-
107 induced die-off to the diversity and structure of soil microbial communities,
108 distinguishing between bacterial and fungal communities;

109 - To assess the role of bacterial and fungal diversity in variables related to
110 soil functioning (soil basal and substrate-induced microbial respiration, microbial

111 metabolic quotient), according to (i) vegetation type, and (ii) climate-induced die-
112 off.

113

114

115 **2. Material and methods**

116

117 *2.1. Study area*

118 The study was performed in the Doñana National Park in south-western Spain (38°13′ N,
119 48°10′ W). The site is located approximately 3 km from the Atlantic Ocean on sandy
120 soils derived from a dune field.The parent material of the studied soil is eolian sandy
121 sediment from Holocene covering gravels and sandy sediments from Pliocene–
122 Pleistocene, constituting a system of stabilized dunes (Siljeström & Clement, 1990). The
123 main soil types are Typic Xeropsamment (dune tops), Aquic Xeropsamment (dune
124 slopes), and Humaqueptic Psammaquent (inter-dune depressions) (Siljeström et al. 1994).
125 Soil texture composition is dominated by sand (Muñoz-Reinoso & García Novo, 2004).

126 The vegetation consists of wetlands, patches of annual grassland, 1-2 m of tall shrubland
127 dominated by Cistaceae (*Halimium halimifolium* (L.) Willk., *Halimium commutatum* Pau,
128 *Cistus libanotis* L.), Fabaceae (*Staurancanthus genistoides* (Brot.) Samp, *Ulex australis*
129 Clemente) and Lamiaceae (*Rosmarinus officinalis* L., *Lavandula stoechas* L., *Thymus*
130 *mastichina* L.), and locally dense stands of *Juniperus phoenicea* L. woodland that are
131 often mixed with *Pinus pinea* L.

132 The climate is sub-humid Mediterranean with oceanic influence. Mean annual rainfall is
133 560 mm, with great between-year variability, ranging from 1,000 mm to less than 300
134 mm. Most rainfall (approx. 80%) falls between October and March while July and
135 August are normally very dry (García Novo, 1979). The mean annual temperature is 16.5
136 ºC, with mean monthly values ranging from 10.0 ºC in January to 24.7 ºC in July. An
137 episode of extreme drought combined with cold temperature occurred in the hydrological

138 year of October 2004 to September 2005, when total rainfall only achieved 173.0 mm,
139 making it the second driest year since 1859 (see Figure S1). Specifically, winter rainfall
140 was only 58.9 mm, less than one third the historical average, while the minimum
141 temperatures in January and February 2005 were 1.1ºC and 0.9ºC, respectively,
142 considerably below mean values (5.0ºC for January and 6.1ºC for February). This episode
143 produced a general die-off of the perennial vegetation, including shrublands and
144 woodland dominated by *J. phoenicea*. Annual rainfall recovered its normal values in the
145 years following this episode, ranging from 468.3 mm in the 2005-2006 hydrological
146 period to 784.2 mm in 2009-2010 (Figure S1).

147

148 *2.2. Field sampling*

149 The study was performed in three localities within an area of approximately 10 km^2:
150 Raposo (N 37º0'2" W6º30'20''), Ojillo (N36º59'45" W6º30'40'') and Marqués (N 37º0'45"
151 W6º31'50"), separated from each other by at least 1 km. The three sites differed slightly
152 in their elevation with respect to the dune field (Raposo was the lowest, at 18 m a.s.l.,
153 Marqués the highest, at 30 m a.s.l., and Ojillo in an intermediate position, close to the top,
154 at 21 m a.s.l) and, as a result, they probably differed in their distance from the water table.
155 They are therefore on a natural gradient of water stress. Four sampling points in Raposo
156 and Ojillo and five sampling points in Marqués were selected for each vegetation type
157 (*Juniperus* woodland, shrubland and grassland) and degree of defoliation - damaged or
158 unaffected - (hereafter, drought impact): damaged and unaffected*Juniperus* woodland,
159 damaged and unaffected shrubland, and grassland (see Figure S2). Soil analyses revealed
160 that there were no significant differences in pH between the different habitats, although
161 conductivity was lower in grassland (Table 1). Sampling points of damaged stands
162 showed percentages of dry canopy higher than 50%. in relation to total vegetation cover
163 (TableS1). Given that annuals dominated the grassland, no distinction could be detected
164 between die-off-damaged and unaffected stands. The four sampling points per vegetation
165 type and degree of defoliation were separated from each other by at least 10 m. In May
166 2010, five years after the drought episode, two 2-m-long perpendicular transects were
167 established at each sampling point; vegetation surveys were then performed by measuring

168 the canopy projection of all the plants above the transects, distinguishing living and dry,
169 recently dead plants (Table 1; see Table S1 for a detailed information of the location and
170 vegetation composition and state at the sampling points). At this point, vegetation had not
171 yet fully recovered on damaged plots (Figure S2), with the percentage of dry canopy with
172 respect to total vegetation cover ranging from 57.5% to 97% (see Table S1; see also
173 Figure S3 for a description of the performance since before 2007 to 2013 of shrubland
174 cover on stands with diferent degrees of die-off damage). These vegetation surveys
175 performed at sampling-point transects were used to estimate plant diversity (H' index
176 calculated for all plant species including grasses and woody species) and the abundance
177 of woody species (estimated by the sum of the percentage cover of the different woody
178 species on thetransects).

179 Soil samples were collected from the upper 10 cm of the soil profile, excluding litter. At
180 each sampling point a circle was drawn, with the two perpendicular transects as diameters.
181 Soil samples were then collected at the centre of the four circle quadrants defined by
182 transects. The four soil collections from each sampling point were pooled to obtain a 150-
183 200 g soil sample, which was sealed in a plastic bag and kept at 4ºC. Twelve replicates
184 were then obtained per vegetation type and die-off treatment, four of which corresponded
185 to each locality. After sampling, the soils were sieved (< 2mm) and homogenized. An
186 aliquot of each sample was stored at -20ºC for molecular analysis and another aliquot was
187 stored at 4ºC until the assays of soil microbial biomass carbon and basal and substrate-
188 induced respiration were performed.

189

190

191 *2.3. Soil Analysis*

192 The percentage of soil organic matter (SOM) was estimated as ignition loss (550°C for 2
193 h) following Nelson & Sommers (1982). Electrical conductivity and soil pH were
194 measured at soil water ratios of 1:5 and 1:2.5, respectively.

195 Microbial biomass carbon in soil was estimated following the fumigation extraction
196 method. Two portions of moist soil (10 g dry weight equivalent) were weighed. In the
197 first (non-fumigated), C was immediately extracted with 40 ml of 0.5 M K_2SO_4 for 30
198 min by shaking, before being filtered (Whatman no. 42). The second was fumigated for
199 24 h at 25 °C with ethanol-free $CHCl_3$ and then C was extracted as described above.
200 Extractable organic C was determined after oxidation with 0.4 M $K_2Cr_2O_7$ at 150 °C for
201 30 min (Vance et al., 1987). Microbial biomass carbon (MBC) was calculated as follows:

202 MBC = EC * 2.64

203 where EC is the difference between organic C extracted from fumigated soil and from
204 non-fumigated soil, and 2.64 is the conversion factor from EC into microbial biomass
205 (Vance et al., 1987).

206 Soil functioning was assessed by focusing on decomposition via several related
207 parameters: Basal Microbial Respiration (BR), Substrate-induced respiration (SIR) and
208 microbial metabolic quotient (qCO_2). BR was determined by placing 30g of soil in
209 hermetic glass jars (1,000 mL) together with 10mL 0.05N NaOH in 20mL glass vials. All
210 samples were incubated for five days at 22.5°C, and the CO_2 that evolved and was
211 trapped during the period was determined by titration of the NaOH with 0.1N HCl
212 (Anderson, 1982). SIR was measured for 12 consecutive hours, adding the amount of
213 glucose necessary for eliciting maximum respiratory activity (2, 5, and 8 g glucose kg^{-1}
214 soil were previously recorded). CO_2 release was measured as described above. qCO_2 was
215 calculated as the ratio between basal respiration and microbial biomass carbon (µg CO_2-C
216 g^{-1} $C_{micr}h^{-1}$).

217 *2.4. DNA extraction and microbial communities fingerprinting by TRFLP analysis*

218 Soil DNA was extracted from soil samples using the MoBio ultraclean DNA soil kit
219 (MoBio, Laboratories Inc., CA), as recommended by the manufacturer.

220 Briefly, the DNA extraction methods involved chemical lysis of microbial cells with
221 gentle bead-beating. Released DNA was bound to a silica spin filter that was
222 subsequently washed, and the DNA was recovered in elution buffer solution. DNA yields

223 and quality were checked after electrophoresis in 0.8% (w/v) agarose gel stained with
224 ethidium bromide under UV light (Sambrook et al., 1989). In some cases we obtained an
225 uninformative TRFLP profile, probably due to a scarce amplification yield; in these cases,
226 samples were discarded for subsequent analyses (see in Table 1 the final number of
227 replicates of each combination of vegetation type and degree of defoliation for both
228 bacteria and fungi).

229 For bacterial communities, 16S rRNA gene from extracted DNA samples was amplified
230 using the universal eubacterial primers: forward primer 8F
231 (AGAGTTTGATCCTGGCTCAG), labelled with fluorescent dye 6FAM (Applied
232 BioSystem), and reverse primer 1398R (ACGGGCGGTGTGTACAAG).

233 Fungal DNA was amplified using the highly conserved fungal rRNA gene primers ITS 1-
234 F (CTTGGTCATTTAGAGGAAGTAA), labelled with 6FAM (Applied BioSystem) and
235 ITSF4 (TCCTCCGCTTATTGATATGC).

236 PCR reaction was performed in 25 μl PCR Master Mix by Promega (containing 50
237 units/mL Taq DNA polymerase in a proprietary reaction buffer, 400 μM each dNTP and
238 3 mM $MgCl_2$), 0.2 μM of each primer, and 1 μl of template DNA.

239 The thermocycling conditions for bacterial DNA were as follows: a hot start at 95°C for 3
240 min (one cycle); 95°C for 30 sec, 58°C for 30 sec, 72°C for 1min (35 cycles), and a final
241 extension at 72°C for 7 min.

242 The thermocycling conditions for fungal DNA were as follows: a hot start at 94°C for 4
243 min (one cycle); 94°C for 1 min, 55°C for 1 min, 72°C for 2min (40 cycles), with a final
244 extension at 72°C for 7 min. PCR products of the correct size (~1380 and 700 bp for
245 bacterial and fungal primers, respectively) were confirmed by 1.5% (w/v) agarose gel
246 electrophoresis and subsequently purified using UltraClean® PCR Clean-Up Kit (MoBio
247 Laboratories Inc., CA), according to the manufacturer's instructions (50 μl final volume).
248 The purified PCR product (30 μl) was then digested with 2U of MspI restriction
249 enzymes (Biolabs) in the manufacturer's buffer (total reaction volume 50 μl) overnight
250 at 37ºC. Excess primers and amplification and digestion reagents were removed by

251 precipitating with 5 μl of 125 mM EDTA and 5 μl of 3M NaOAc (pH5.2) and 125 μl
252 100% ethanol. After centrifugation, the pellet was washed in 70% ethanol, dried and
253 suspended in 10 μl of deionized formamide and 0.25 μl of size standard GeneScan 500-
254 Rox (Applied Biosystem) was added. Samples were denatured for 4 minutes at 95º and
255 analyzed using an automated sequencer ABI Prism 3100 Genetic Analyzer (Applied
256 Biosystem). Size and intensity of data were assessed using Peak Scanner software
257 (Applied Biosystem). For bacteria, peaks corresponding to the range of 50 to 700 bp were
258 considered, and for fungi this range was 50 to 500 bp. Noise filter was carried out with
259 the online software T-REX (Culman et al., 2009): only peaks with a height exceeding a
260 value equivalent to the standard deviation (assuming zero mean) of the whole set of peaks
261 were included in further analysis (Abdo et al., 2006). From our experience, and according
262 to criteria used in previous studies using ABI 3100 (and the preceding version), the error
263 in determining fragment sizes with our ABI 3100 automated DNA sequencer was less
264 than 0.5 bp (Curiel Yuste et al., 2011), and typically less than 0.2 bp. For this reason we
265 use criteria of 0.5bp alignment. The remaining TRFs in different profiles varying in size
266 by 0.5 bp or less were considered the same and were aligned using the moving average
267 algorithm of the software program T-REX (Culman et al., 2009). TRF do not strictly
268 correspond to taxa but they are recognized as providing a consistent proxy for estimating
269 structural characteristics of the microbial communities (f.e. Curiel Yuste et al., 2011;
270 Storey et al., 2014). Therefore, the soil TRF richness of each sample was calculated as the
271 total number of distinct TRF sizes in a profile, and H' and evenness diversity indexes
272 (Magurran, 2004) were calculated considering peak area as an estimation of abundance.
273 The evenness index was calculated as the quotient between the observed H' and the $\log_2$
274 of richness.

275

276 *2.5. Data analysis*

277 General linear models (GLMs) were used to analyze factors contributing to (1) soil
278 microbial diversity and composition, and (2) parameters describing microbial community
279 functioning (Table S2).

280 Microbial diversity was estimated separately for bacteria and fungi using richness,
281 evenness and H' as response variables. Microbial community composition was
282 characterized by PCA (uncentered and unscaled) carried out with the values of the TRF
283 peaks. The first axis of these PCA was used as an overall description of microbial
284 communities. Only peaks that were present in at least 20% of the samples (259 out a total
285 of 637 in bacteria, and 48 out a total of 456 in fungi) were considered. This ordination
286 was appropriate, given the short environmental gradient considered and the selection of
287 frequent TRF peaks (Leps & Smilauer, 2003).

288

289 Functioning of the microbial community was analyzed using: basal microbial respiration
290 (BR), substrate-induced microbial respiration (SIR), and microbial metabolic quotient
291 (qCO_2) as response variables.

292 Two sets of GLM were performed: the first addressed the relationship between soil
293 microbial communities and vegetation in unaffected areas (hereafter, vegetation model),
294 (see Field Sampling for a more detailed description) and the second aimed to analyze the
295 effect of the die-off (hereafter, die-off model). The full combination of vegetation types
296 (*Juniperus* woodland, shrubland and grassland) and die-off (unaffected, damaged) could
297 not be built because no effect of the drought episode was observable on grassland at the
298 sampling time. Thus, the vegetation model considered the three types of vegetation, while
299 the drought model did not include grassland. In addition to vegetation type and die-off,
300 the explicative variables included in the models were plant diversity (H'), abundance of
301 woody species (estimated by the sum of the percentage cover of the different woody
302 species on the transects), soil organic matter (SOM) and location. In the vegetation model,
303 the interaction between vegetation type and SOM was included. In the die-off model, the
304 interactions between vegetation type and die-off and SOM, and the interactions between
305 die-off and SOM were also included (see Table S2 for a detailed description of models).
306 Since vegetation type and plant species diversity are correlated, GLM that excluded plant
307 species diversity while maintaining vegetation type as an explicative variable were also
308 performed.

309

310 The analysis of the functioning parameters was similar but included as explicative
311 variables vegetation type, bacterial and fungal diversity, interactions between bacterial
312 and fungal diversity and vegetation type, SOM, microbial biomass carbon, and location in
313 the vegetation model. Different models were built for the three different indicators of
314 microbial diversity: richness, evenness, H'. In the die-off models, die-off and its
315 respective interactions with bacterial and fungal diversity and vegetation type were
316 included. Microbial biomass carbon was excluded from the models analyzing microbial
317 metabolic quotient since these variables are inversely correlated.

318 In these GLM, fungal richness, abundance of woody species, BR, SIR, qCO_2, SOM, and
319 microbial biomass carbon were log-transformed to attain normality. Model selection was
320 carried out through a step-wise selection procedure using the Aikake information
321 criterion (AIC) to select the best-fitted models. No-significant factors (high p-values)
322 remained in the models when their interaction with other factors was significant.
323 Categorical variables were coded hierarchically after splitting the levels into two groups,
324 as indicated by brackets and the successive significant variables consisting of each set of
325 two-groups were then included in the model. All the GLM analyses were performed
326 using JMP 10.0 (SAS Institute Inc.).

327 Differences in pH, conductivity, SOM and microbial biomass carbon between the five
328 vegetation categories, including damaged stands of *Juniperus* woodland and shrubland,
329 were estimated by one-way ANOVA and post-hoc pairwise t-tests. All these variables
330 were log-transformed to attain normality.

331

332

333 **3. Results**

334

335 *3.1. Bacterial community*

336 More bacterial TRFs were obtained than fungal ones in all the habitats (Figure 1). In
337 unaffected stands, bacteria richness was explained in models by increasing plant diversity
338 and decreasing SOM (Figure 2, Table S3). This effect of plant diversity was strongly
339 related to vegetation type, because in the GLM that excluded plant diversity, vegetation
340 type exhibited a significant effect while AIC retained a similar value (Table S3). The
341 highest bacterial richness was found in grasslands – the habitat with the highest
342 vegetation diversity (one-way ANOVA, $F = 41.53$, $p < 0.001$) - and the lowest in
343 *Juniperus* woodland (Figure 1). Bacterial evenness was also determined by plant
344 diversity and vegetation type (Table S3). In this case the relationship was negative: the
345 lowest evenness was found in grassland and the highest in *Juniperus* woodland (Figure 1).
346 Accordingly, bacterial H' was determined by vegetation type, with the highest values in
347 grassland and the lowest in *Juniperus* woodland (Figure 1). Bacterial composition,
348 estimated from the first axis of PCA, was determined by vegetation type in unaffected
349 stands and by die-off in damaged ones (Table S4). Accordingly, the PCA scores of plots
350 demonstrated distinct values for unaffected and damaged stands of *Juniperus* woodland
351 (Figure 3).

352 In damaged stands, bacterial richness also related positively to plant diversity and
353 negatively to SOM (Figure 2; Table 4). Die-off had a significant effect on bacterial
354 richness in the model excluding vegetation diversity (Table 2): richness increased in
355 damaged stands, particularly in *Juniperus* woodland (Figure 1). This increase concurred
356 with higher vegetation richness in affected plots of *Juniperus* woodland (Table 1).
357 Bacterial evenness was negatively determined by vegetation diversity, and in the model
358 excluding vegetation diversity the significant factors were vegetation type (with lowest
359 values in shruband), die-off (with lowest values in damaged stands), and locality (Table
360 2).

361

362 *3.2. Fungal community*

363 Fungal diversity was better explained by our models (between 30 and 53%) than bacterial
364 diversity (less than 30%). In unaffected stands, fungal richness was higher in *Juniperus*

365 woodland (Figure 1). Fungal richness displayed an overall humped relationship with
366 SOM (polynomial fit to degree 2, $r^2 = 0.200$, $p = 0.005$) (Figure 4a). Accordingly, there
367 was a significant interaction between vegetation type and SOM (Table S4): fungal
368 richness increased linearly with SOM in grassland ($r^2 = 0.750$, $p = 0.012$) while there was
369 no significant correlation in shrubland ($r^2 = 0.110$, $p = 0.268$) and *Juniperus* woodland (r^2
370 $= 0.001$, $p = 0.950$) (Figure 4a). Fungal evenness was also determined by vegetation type,
371 with lower values in *Juniperus* woodland (Figure 1, Table S4). Fungal H' showed a
372 relationship with SOM similar to that of richness (Figure 4b, Table S5) (polynomial fit to
373 degree 2, $r^2 = 0.139$, $p = 0.028$; correlation between fungal H' and SOM in grassland: $r^2 =$
374 0.562, $p = 0.052$, in shrubland, $r^2 = 0.174$, $p = 0.156$, in *Juniperus* woodland, $r^2 = 0.084$, p
375 $= 0.486$).

376 In damaged stands, there was a significant interaction between vegetation type and die-
377 off in models analyzing fungal richness (Table 3). Fungal richness decreased with die-off
378 in *Juniperus* woodland, but not in shrubland (Figure 1). There was also a significant
379 interaction between die-off and SOM (Table 3): fungal richness was positively but
380 marginally related to SOM in damaged stands ($r^2 = 0.139$, $p = 0.079$) but not in
381 unaffected ones ($r^2 = 0.082$, $p = 0.209$) (Figure 4a). Models analyzing evenness also
382 showed a significant interaction between vegetation type and die-off: evenness increased
383 with die-off in *Juniperus* woodland and decreased in shrubland. There was also a
384 significant interaction between die-off and SOM (Table 3): H' increased significantly
385 with SOM in damaged stands ($r^2 = 0.298$, $p = 0.007$) but not in unaffected ones ($r^2 =$
386 0.087, $p = 0.193$) (Figure 4b).

387 The first and second axis of the PCA ordinations of fungal composition explained 36.8%
388 and 8.6% of the TRF peak variability, respectively. The GLM of fungal composition
389 estimated by the first PCA axis were poor and did not attain statistical significance.

390

391 *3.3. Soil microbial functional properties*

392 In unaffected stands, microbial respiration (BR, SIR) was explained by locality,
393 vegetation type, and bacterial diversity. SIR was negatively correlated to bacterial

394 diversity (H' index) ($r^2 = 0.423$, $p = 0.016$) in grassland, explaining the observed
395 significant interaction between vegetation type and bacterial evenness (Table S6).

396 In damaged stands, SIR was 1.8 (in *Juniperus* woodland) and 1.3 (in shrubland) times
397 higher than in unaffected ones. Die-off increased BR 1.4 times in *Juniperus* woodland
398 and 1.8 times in shrubland (Tables 1, 4). Accordingly, qCO_2 was also higher in damaged
399 stands (Tables 1, 4), particularly in shrubland, but it was not related to microbial diversity.
400 Both BR and SIR were negatively correlated with bacterial richness and H'. There were
401 significant interactions between bacterial evenness and vegetation type and die-off in the
402 respiration models (Table 4).

403

404

405 **4. Discussion**

406

407 *4.1. Microbial diversity and vegetation in unaffected plots*

408 This study corroborated the strong ecological relationship between soil microbial
409 communities and vegetation and showed how this relationship was modified by abrupt
410 weather-induced alterations to the environment. In the absence of die-off, the type of
411 vegetation was an important determinant of the diversity and structure of soil microbial
412 communities. Both processes resulting directly from die-off (i.e. litter fall) and from the
413 following successional replacements (i.e. increase in grasslands species in woody
414 vegetation) contributed to the explanation of the response of soil microbial communities
415 to the climatic events.

416 Several papers have identified changes in soil microbial communities between vegetation
417 units (Balser & Firestone, 2005; CurielYuste et al., 2012) or biomes (Fierer et al., 2009;
418 Lauber et al., 2009), and our study corroborated these findings on a small scale between
419 vegetation types coexisting in the same landscape (see also Cregger et al., 2012).
420 Nevertheless, bacterial and fungal diversity responded differently. Under unaffected
421 canopies there was a gradient towards lower bacterial richness from grassland to
422 *Juniperus* woodland, which coincided with a similar gradient in plant diversity. The

423 opposite trend in bacterial evenness pointed to changes in the hierarchical structure of the
424 bacterial community, with a large number of rare taxa in more diverse plant communities.
425 Conversely, fungal richness was clearly higher and evenness was lower under *Juniperus*
426 woodlands. These results concurred with observations of soil microbial communities in
427 the gradient from grasslands to forest (e.g. Imberger & Chiu, 2000; Kageyama et al.,
428 2008).

429 The observed association of bacterial and vegetation diversity can be explained by the
430 ability of plant species to promote different niches for bacteria. Microhabitats under
431 *Juniperus* woodland were more homogeneous with respect to grasslands, due to a dense
432 canopy that prevented the establishment of other plant species, thus reducing plant
433 richness (Table 1). The differential quality of the substrates provided by plants in these
434 vegetation types is also expected to play a critical role. As in other gymnosperms,
435 *Juniperus* foliage is rich in monoterpenes and other secondary compounds that have been
436 reported to constrain the development of a rich soil microbiota (Montane et al., 2010;
437 Zhang et al., 2013). Indeed, the decrease in bacterial richness as SOM accumulates in
438 soils (from grassland to shrubland and *Juniperus* woodland) suggested that bacteria might
439 be sensitive to SOM quality, assuming that this accumulation was partly caused by
440 decreasing degradability of the substrate.

441 In contrast, the variation in fungal communities, which was better explained by our
442 models than the bacterial one, appeared to be mainly driven by the properties derived
443 from the different vegetation types rather than by plant diversity itself. This is particularly
444 true of the microhabitat created by *Juniperus* woodlands, where fungal composition and
445 diversity were differentiated, particularly in unaffected stands (Cregger et al., 2012). The
446 richer and more structured fungal community (less evenness) under *Juniperus*
447 woodlands could be explained by higher levels of mycorrhization under this woody
448 vegetation (Williams et al., 2013). Moreover, the typical recalcitrance of SOM under
449 woody vegetation stands, resulting in high C:N ratios, may have favored the growth of a
450 diverse saprophytic fungal community able to decompose these molecules under
451 *Juniperus* woodland(Fierer et al., 2009).

452

453 *4.2. Climate-induced die-off and microbial diversity*

454

455 The weather anomaly affected soil microbial diversity through its negative impact on
456 vegetation health and the associated secondary succession. Nevertheless, the bacterial and
457 fungal communities again responded differently. The positive effect of die-off on
458 bacterial richness was largely due to the colonization of the space formerly occupied by
459 dead *Juniperus* by grasses and herbs, which were richer in plant species. This
460 interpretation is supported by models including vegetation diversity, and by the
461 convergence between bacterial composition under damaged *Juniperus* woodland and
462 under shrublands and grasslands (see first axis in Figure 3a). This further confirmed other
463 previous observations highlighting the rapid readjustment of soil bacterial communities to
464 changes in vegetation associated with tree mortality (Curiel Yuste et al., 2012; Barba et
465 al., 2013). It also suggested that soil bacterial communities are good indicators of below-
466 ground colonization processes occurring during secondary succession. The increased
467 richness in die-off vegetation was accompanied by lower evenness, particularly in
468 *Juniperus* woodland, which corresponds to a higher number of dominant and/or rare taxa
469 (Deslippe et al., 2012).

470 In contrast, the response of fungal communities to the weather-induced die-off resulted
471 in a reduction in richness only in defoliated *Juniperus* woodlands, probably due to the
472 dependence of the mycoflora on the productivity and health of the *Juniperus* individuals
473 (Hogberg et al. 2009, Williams et al., 2013). Other studies have also shown that fungal
474 soil communities are generally less sensitive to seasonal drought conditions than bacterial
475 ones (Curiel Yuste et al. 2011; Barnard et al. 2013). Unlike undamaged stands, the
476 positive relationship between SOM and fungal richness in die-off stands suggested that
477 the scarcity of this resource (SOM) in damaged shrublands (Table 1) might have
478 negatively affected the saprophytic fungal community.

479

480 *4.3. Soil functional properties: respiration and microbial activity*

481 Overall, microbial respiration and microbial metabolic activity were better explained in
482 our models than the indicators of soil microbial diversity and structure. The functional
483 properties of the microbial community were therefore more predictable than the structural
484 properties, as they are more directly linked to physical-chemical variables, such as
485 temperature, water availability, and substrate, i.e. SOM decomposition (Curiel Yuste et
486 al., 2007).

487

488 In unaffected stands, indicators of microbial metabolic activity (microbial BR, SIR and
489 qCO_2) were mainly explained by vegetation type, together with local effects. Grassland
490 displayed higher BR, SIR, and qCO_2 than woody-dominated vegetation (Table 1),
491 probably because the tissues produced by herbaceous species had lower proportions of
492 lignin and lower C:N or C:P ratios than woody plants (Montane et al., 2010; Zhang et al.,
493 2013), which makes SOM more degradable for microbial consumption. Furthermore, soil
494 samples were collected when herbacous species were at their productive peak, and
495 probably when labile exudate production was at its maximum (Curiel Yuste et al., 2007).
496 Probably as the result of this high metabolic activity of microbial communities, SOM
497 further tended to accumulate less under grassland (Table 1). This higher microbial
498 metabolic activity under grasslands coincided with higher bacterial richness, as explained
499 above. However, the overall statistical relationship between bacterial diversity and
500 microbial functioning was modest, interacting in some cases with vegetation type, and
501 suggesting that in general microbial functioning was somewhat uncoupled from microbial
502 diversity. This was further supported by the fact that fungal richness did not help to
503 explain variability in any of the indicators of microbial functioning, even though other
504 studies have found that fungal biomass is related to SOM decomposition (CurielYuste et
505 al., 2011),

506

507 Die-off also resulted in changes in the functioning of the microbial communities, as
508 reflected in the increased rates of BR, SIR, and qCO_2. These results supported previous
509 studies in which drought-induced die-off resulted in higher soil metabolic activity,

510 probably associated with an increase in the labile C pool (Curiel Yuste et al., 2012; Barba
511 et al., 2013). We hypothesize that these effects of die-off on soil functioning could be
512 attributable, at least partially, to the litter increase after defoliation, which remained for
513 several years. The increase in microbial metabolic activity was not associated with an
514 increase in the microbial biomass (microbial biomass carbon) but rather with a shift in the
515 structure and composition of the microbial community. We hypothesize that this shift
516 would involve an increase of pioneer,*r* strategy-like microorganisms, with high turnover
517 rates, with respect to K strategy-like microorganisms.

518

519 Die-off further strengthened the relationship between the structural characteristics of soil
520 microbial communities, particularly bacteria, and their functioning, as reflected in the
521 significant role played by bacteria in predicting variations in soil metabolic activity. This
522 indicated that changes in soil C stocks associated with die-off could be partly caused by
523 the structural and functional adjustments experienced by microbial communities.
524 Therefore, although the contribution of microbial communities in our statistical models
525 remained lower than the effect of die-off itself, this study provided novel evidence of the
526 direct relationship between microbial diversity and soil CO_2 fluxes. Thus, any models
527 considering soil C dynamics under strong weather-driven ecological perturbations and
528 subsequent succession should consider the ecology of microbes, particularly bacteria, and
529 their ecological relationships with plants.

530

531 *4.4. Conclusions*

532 Bacterial and fungal communities showed contrasting patterns in relation to the diversity
533 of vegetation and soil C properties (Zak et al., 2013). There was a gradient of decreasing
534 SOM quality, reflected in lower BR, SIR, and qCO_2, from grassland to *Juniperus*
535 woodland, limiting the richness of bacterial communities. This SOM gradient coincided
536 with a decrease in plant diversity that provided niches to the bacterial guild. In contrast,
537 fungal communities increased their relevance in terms of richness in *Juniperus* woodland,
538 and we attribute this finding to their ability to consume less degradable SOM.

539 Our study revealed that weather anomalies produced effects on microbial communities
540 that persisted for several years after the weather event, in contrast with the resilience of
541 soil microbial communities observed in experimental climate manipulations (Cruz-
542 Martinez et al., 2009; Curiel Yuste et al., 2014). This delayed effect on microbial soil
543 communities was associated with the remaining impact of vegetation die-off, involving
544 changes in vegetation composition and soil properties, and corresponded with
545 observations from Mediterranean forests that have experienced drought-induced die-off
546 (Curiel Yuste et al*.,* 2012). As regards the bacterial guild, the drought event did indeed
547 act as a typical disturbance, promoting a pulse of diversity by generating new habitats,
548 increasing resource availability, or releasing competition. In contrast, in the fungal guild,
549 the effect of the episode was more sensitive to habitat modification, in our case that of the
550 *Juniperus* woodlands, which was largely determined by the fate of the dominant species.

551

552 In conclusion, this study revealed how the impacts of climate change on vegetation
553 spread to soil microbial communities. Thus, weather events have the potential to produce
554 relevant changes in ecosystem functioning by modifying the functional properties of soils
555 through changes in soil microbial communities. Given that current climatic projections
556 point to an increase in episodes of climatic anomalies that could result in vegetation shifts
557 (Lloret et al., 2012), these cascade effects merit further attention. In addition to the
558 expected modification of microhabitats (radiation, and consequently temperature and
559 humidity), the mechanisms involved in the climatic modification of the relationship
560 between vegetation and the soil microbial community include changes in litter quantity
561 and quality as well as changes in rhizosphere - and related exudates -, which are
562 determined by standing vegetation.

563

564

565

566

5. Acknowledgements

This study was supported by the Spanish Ministry of Education and Sciences (projects CGL2009-08101, CGL2010-16373, CGL2012-32965), by the Ministry of Economy and Competitiveness (CGL2013-42271), by the Government of Catalonia (AGAUR grants 2009-SGR-00247) and by the ICTS-Reserva Biológica de Doñana (projects 38/2007, 27/2009, and 11/2013) and by the Goverment of Madrid (REMEDINAL 2; CM S2009 AMB 1783). Personnel from the ICTS-RBD kindly supported the fieldwork. JCY acknowledges the support of the "Ramon y Cajal" program.

576 **References**

577 Abdo Z, Schuette UME, Bent SJ, Williams CJ, Forney LJ, Joyce P (2006) Statistical
578 methods for characterizing diversity of microbial communities by analysis of terminal
579 restriction fragment length polymorphisms of 16S rRNA genes. *Environ Microb* 8: 929-
580 938.
581
582 Allen CD, Macalady AK, Chenchouni H et al. (2010 A global overview of drought and
583 heat-induced mortality reveals emerging climate change risks for forests. *Forest Ecol*
584 *Manag 259*: 660–684.
585
586 Anderson JPE (1982) Soil respiration. In: *Methods of soil analysis. Part 2. Chemical and*
587 *Microbiological Properties. 2nd ed*. (Page AL, ed). , pp. 831-871. Agron. Monogr. 9.
588 ASA and SSSA, Madison, WI..
589
590 Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in
591 rhizosphere interactions with plants and other organisms. *Ann Rev Plant Biol* 57: 234–
592 266.
593
594 Balser TC, Firestone MK (2005) Linking microbial community composition and soil
595 processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73:
596 395-415.
597

598 Barba J, Curiel Yuste J, Martinez-Vilalta J, Lloret F (2013) Drought-induced substitution
599 is reflected in the spatial distribution of soil CO_2 effluxes of a mixed Mediterranean forest.
600 *Forest Ecol Manag* 306: 79-87.
601
602 Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change
603 through carbon cycle feedbacks. *ISME J* 2: 805–814.
604
605 Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal
606 communities to extreme dessication and rewetting. *ISME J* 7: 2229-2241.
607
608 Berard A, Bouchet T, Sevenier G, Pablo AL, Gros R (2011) Resilience of soil microbial
609 communities impacted by severe drought and high temperature in the context of
610 Mediterranean heat waves. *Europ J Soil Biol* 47: 333-342.
611
612 Cregger M, Schadt CW, McDowell NG, Pockman WT, Classena AT (2012) Response of
613 the soil microbial community to changes in precipitation in a semiarid ecosystem. *Appl*
614 *Environ Microb*78: 8587–8594.
615
616 Cruz-Martinez K, Suttle KB, Brodie EL, Power ME, Andersen GL, Banfield JF (2009)
617 Despite strong seasonal responses, soil microbial consortia are more resilient to long-term
618 changes in rainfall than overlying grassland. *ISME J* 3: 738-744.
619

620 Culman S, Bukowski R, Gauch H, Cadillo-Quiroz H, Buckley D (2009) T-REX: software
621 for the processing and analysis of T-RFLP data BMC. *Bioinformatics* 10: 171.
622
623 Curiel Yuste J, MissonL, Wong S, Goldstein A, Baldocchi D (2007)Response of soil
624 microbial decomposition to environmental changes at two contrasting Mediterranean
625 ecosystems. *Glob Change Biol* 13: 2018-2035.
626
627 Curiel Yuste J, Peñuelas J, Estiarte M, Garcia-Mas J, MattanaS, Ogaya R,
628 Pujol M, Sardans J (2011) Drought-resistant fungi control soil organic matter
629 decomposition and its response to temperature. *Glob Change Biol*17: 1475-1486.
630
631 Curiel Yuste J, Barba J, Fernandez-Gonzalez AJ, Fernandez-Gonzalez AJ, Fernandez-
632 Lopez M, Mattana S, Martínez-Vilalta J, Nolis P, Lloret F (2012) Changes in
633 metagenomical soil bacterial community: diversity, composition and function triggered
634 by drought-induced gap succession preceded changes in soil C stocks and quality. *Evol*
635 *Ecol* 2: 3016-3031.
636
637 Curiel Yuste J, Fernandez-Gonzalez AJ, Fernandez-Lopez M, Ogaya R, Peñuelas J,
638 Sardans J, Lloret F (2014) Strong functional stability of soil microbial communities under
639 semiarid Mediterranean conditions and subjected to long-term shifts in baseline
640 precipitation. *Soil Biol Biochem* 69: 223-233.
641

642 Del Cacho M, Lloret F (2012)Resilience of Mediterranean shrubland to severe drougth
643 episode: the role of seed bank and seedling establishment. *Plant Biol* 14: 458–466.
644

645 Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the
646 composition of Arctic soil microbial communities. *FEMS Microb Ecol* 82: 302-315.
647

648 Eskelinen A, Stark S, Mannisto M (2009) Links between plant community compositin,
649 soil organic matter quality and microbial communities in contrasting tundra habitats.
650 *Oecologia* 161: 113-123.
651

652 Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns
653 in belowground communities. *Ecol Let* 12: 1238-1249.
654

655 Fisher M (1997) Decline in the juniper woodlands of Raydah Reserve in southwestern
656 Saudi Arabia: a response to climate change? *Glob Ecol Biog Let* 6: 379-386.
657

658 Floyd ML, Clifford M, Cobb NS, Hanna D, Delph R, Ford P, Turner D (2009)
659 Relationship of stand characteristics to drought-induced mortality in three Southwestern
660 piñon–juniper woodlands. *Ecol Appl* 19: 1223-1230.
661

662 García Novo F, (1979) The ecology of vegetation of the dunes in Doñana National Park
663 (South-West Spain). In: *Ecological processes in coastal environments* (Jefferies RL,
664 Davy AJ, eds), pp. 571-592. Blackwell, London.

665 Högberg P, Löfvenius MO, Nordgren A, (2009) Partitioning of soil respiration into its
666 autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce
667 forest. *Forest Ecol Manag* 257: 1764–1767.
668

669 Imberger KT, Chiu CY (2001) Spatial changes of soil fungal and bacterial biomass from
670 a sub-alpine coniferous forest to grassland in a humid, sub-tropical region. *Biol Fertility*
671 *Soils* 33: 105-110.
672

673 IPCC (2013) *Climate change 2013: The physical scientific basis*. WMO, UNEP.
674

675 Kageyama SA, Posavatz NR, Waterstripe KE, Jones SJ, Bottomley PJ, Cromack K,
676 Myrold DD (2008) Fungal and bacterial communities across meadow-forest ecotones in
677 the western Cascades of Oregon. *Can J Forest Res* 38: 1053-1060.
678

679 Kennedy N, Edwards S, Clipson N (2005) Soil bacterial and fungal community structure
680 across a range of unimproved and semi-improved upland grasslands. *Microb Ecol* 50:
681 463-473.
682

683 Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of
684 soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl*
685 *Environ Microb* 75: 5111-5120.
686

687 Leps J, Smilauer P (2003) *Multivariate analysis of ecologica data using CANOCO*.
688 Cambridge Univ. Press, Cambridge.
689
690 Lloret F,Siscart D,Dalmases C (2004) Canopy recovery after drought dieback in holm-
691 oak Mediterranean forests of Catalonia (NE Spain). *Glob Change Biol* 10: 2092-2099.
692
693 Lloret F, Escudero A, Iriondo JM, Martinez-Vilalta J, Valladares F (2012) Extreme
694 climatic events and vegetation: the role of stabilizing processes. *Glob Change Biol* 18:
695 797-805.
696
697 Meisner A, De Deyn GB, de Boer W, van der Putten WH (2013) Soil biotic legacy
698 effects of extreme weather events influence plant invasiveness. *Proc Nat Acad Sci*
699 *USA*110: 9835-9838.
700
701 Magurran AE (2004) *Measuring Biological Diversity*. Blackwell, Malden, MA.
702
703 Mitchell RJ, Hester AJ, Campbell CD, Chapman SJ, Cameron CM, Hewison RL, Potts
704 JM (2012) Explaining the variation in the soil microbial community: do vegetation
705 composition and soil chemistry explain the same or different parts of the microbial
706 variation? *Plant Soil* 351: 355-362.
707

708 Montane F, Romanya J, Rovira P, Casals P (2010) Aboveground litter quality changes
709 may drive soil organic carbon increase after shrub encroachment into mountain
710 grasslands. *Plant Soil* 337:151-165.
711
712 Muñoz-Reinoso JC, García Novo F (2004) Multiscale control of vegetation patterns: the
713 case of Doñana (SW Spain). *Landscape Ecol* 20: 51-61.
714
715 Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In:
716 *Methods of soil analysis. Part 2. Chemical and Microbiological Properties. 2nd ed*. (Page
717 AL, ed), pp. 539–579. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
718
719 Sagova-Mareckova M, Omelka M, Cermak L, Kamenik Z, Olsovska J, Hackl E, Kopecky
720 J, Hadacek F (2011) Microbial communities show parallels at sites with distinct litter and
721 soil characteristics. *Appl Environ Microb* 77: 7560-7567.
722
723 Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual, 2nd*
724 *ed*., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
725
726 Sheik CS, Beasley WH, Elshahed MS, Zhou X, Juo Y, Krumholz LR (2011) Effect of
727 warming and drought on grassland microbial communities. *ISME J* 5: 1692–1700.
728
729 Siljeström P, Clement L (1990) Geomorphology and soil evolution of a moving dune
730 system in SW Spain (Doñana National Park). *J Arid Environ* 18: 139–371.

731

732 Siljeström PA, Moreno A, García LV, Clemente LE (1994) Doñana National Park
733 (southwest Spain): geomorphological characterization through a soil-vegetation study. *J*
734 *Arid Environ* 26: 315–323.
735

736 Storey S, Ashaari MM, McCabe G, Harty M,. Dempsey R, Doyle O, Clipson N, Doyle
737 EM (2014) Microbial community structure during fluoranthene degradation in the
738 presence of plants. *J Appl Microbiol 117*: 74-84.
739

740 Ushio M, Wagai R, Balser TC, Kitayama K (2008) Variations in the soil microbial
741 community composition of a tropical montane forest ecosystem, does tree species matter?
742 *Soil Biol Biogeochem* 40: 2699-2702.
743

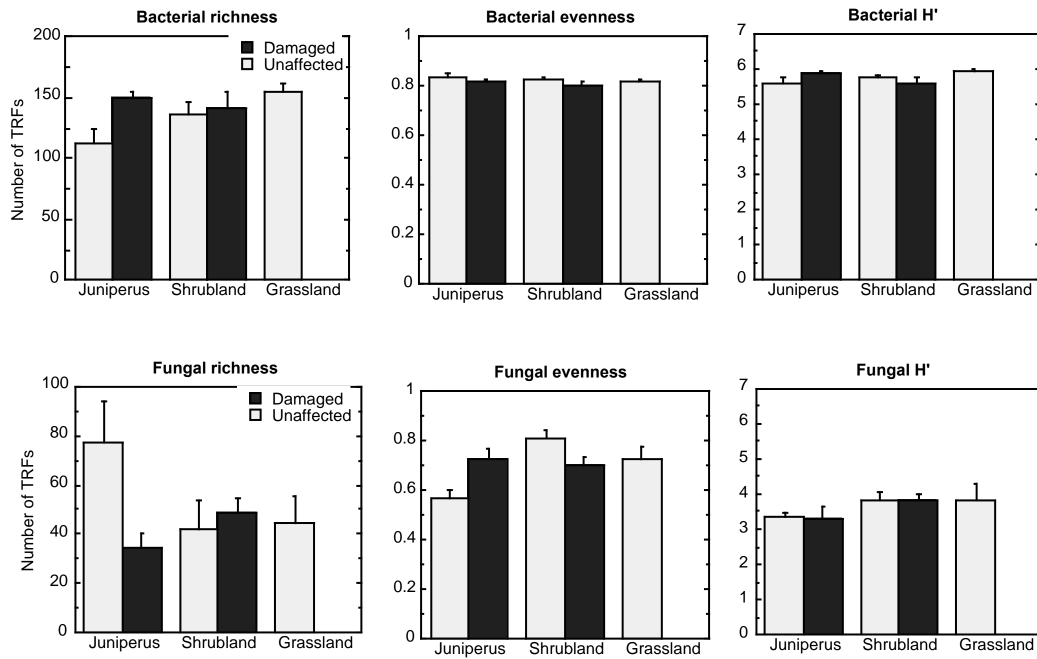
744 Van Mantgem PJ, Stephenson NL, Byrne JC et al. (2009) Widespread increase of tree
745 mortality rates in the Western United States. *Science* 323: 521-524.
746

747 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil
748 microbial biomass-C. *Soil Biol Biogeochem* 19: 703-707.
749

750 Waldrop MP, Firestone MK (2006) Response of microbial community composition and
751 function to soil climate change. *Microb Ecol* 52: 716-724.
752

753 Waldrop MP, Zak DR, Blackwood C, Curtis CD, Tilman D (2006) Resource availability
754 controls fungal diversity across a plant diversity gradient. *Ecol Let* 9: 1127-1135.
755
756 Williams RJ, Hallgren SW, Wilson GWT, Palmer MW (2013) Juniperus virginiana
757 encroachment into upland oak forests alters arbuscular mycorrhizal abundance and litter
758 chemistry. *Appl Soil Ecol* 65: 23-30.
759
760 Zak DR, Holmes WE, White DC, Peacock AD, Tilman D (2003) Plant diversity, soil
761 microbial communities, and ecosystem function: Are there any links? *Ecology* 84: 2042-
762 2050.
763
764 Zhang K, Cheng X, Dang H, Ye C, Zhang Y, Zhang Q (2013) Linking litter production,
765 quality and decomposition to vegetation succession following agricultural abandonment.
766 *Soil Biol Biogeochem* 57: 803-813.
767
768 Zhili He, MX, Deng Y, Kang S, Kellogg L, Wu L,Van Nostrand JD, Hobbie SE, Reich
769 PB, Zhou J (2010) Metagenomic analysis reveals a marked divergence in the structure of
770 belowground microbial communities at elevated CO_2. *Ecol Let*13: 564-575.
771
772
773
774 Figure Legends
775
776

777

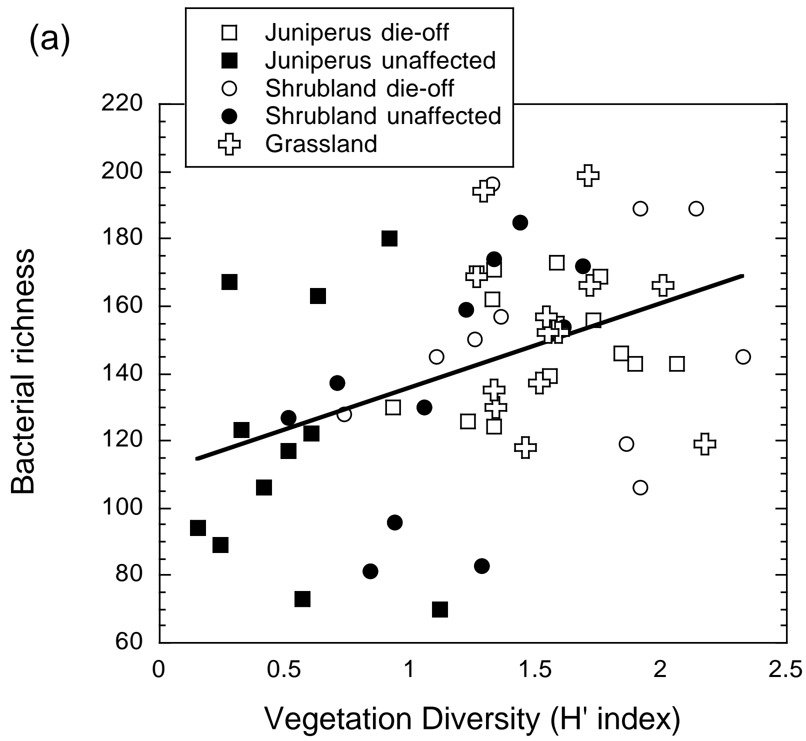


778
779
780
781
782
783 Figure 1.
784
785
786
787
788

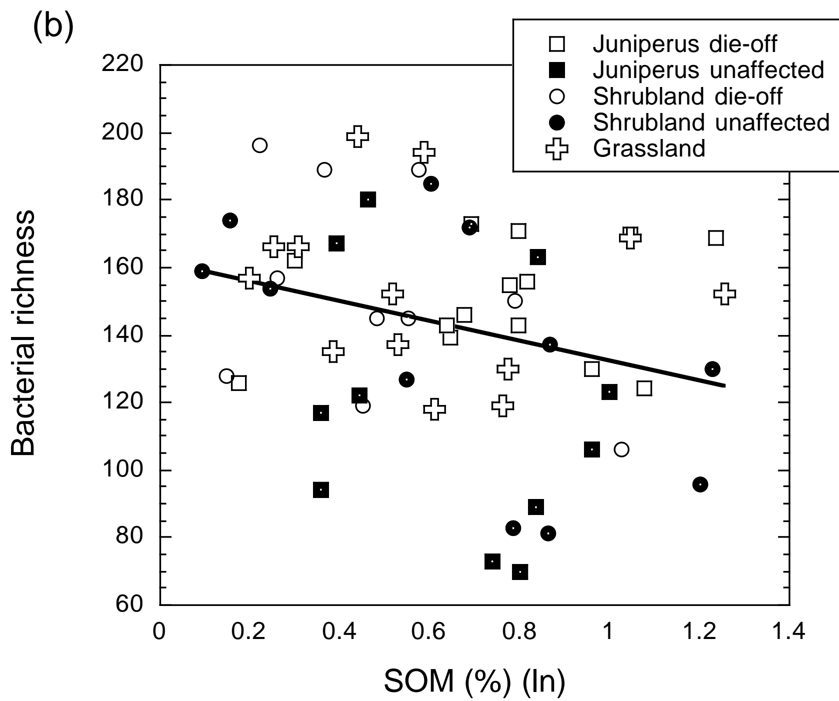
789

790 Figure 1. Mean (SE) bacterial and fungal diversity in different vegetation types and with
791 different climate-induced die-off.

792
793



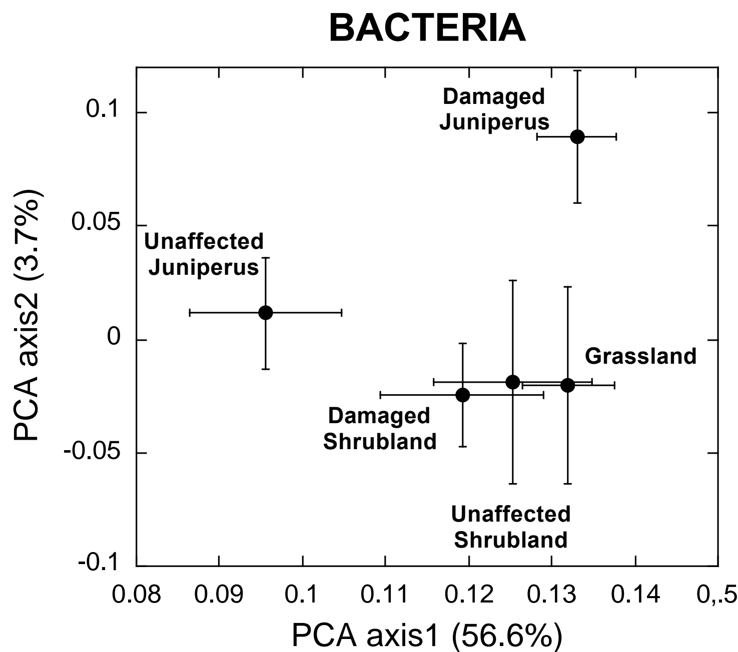
794
795



796 Figure 2
797

798 Figure 2. Relationship between bacterial richness (number of TRF peaks) and (a)
799 vegetation diversity estimated as the H' index, and (b) SOM (%) in the five studied
800 habitats. Linear fit for the whole set of data is shown in (a) (r^2=0.18, p = 0.001) and (b)
801 (r^2=0.08, p = 0.030).

802
803
804
805
806



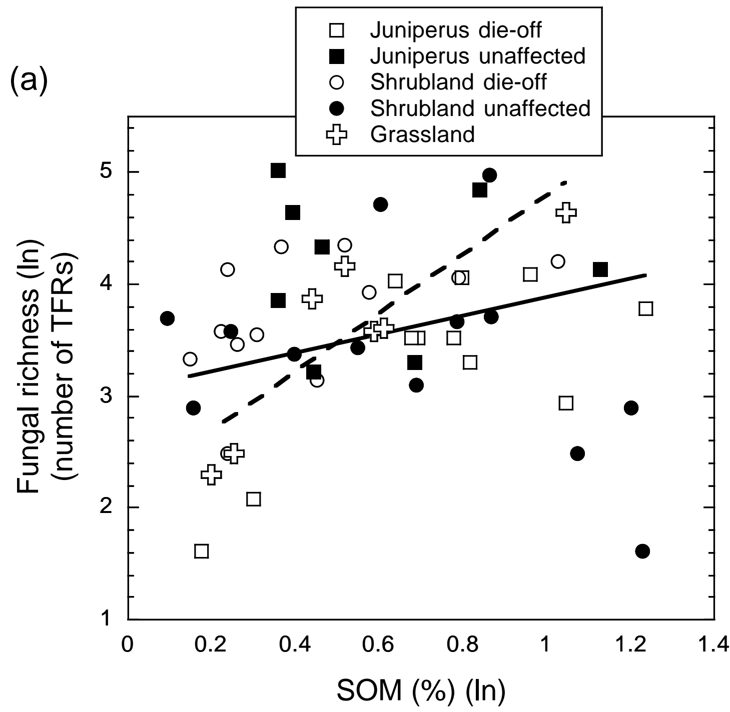
807
808
809
810
811
812 Figure 3.
813

814 Figure 3. Mean coordinates of the first and second axes of PCAs performed for bacteria
815 TRFs peaks (log transformed) from the samples of each vegetation type. Error bars
816 indicate SE. Percentage of variability explained by each axe is given in brackets.

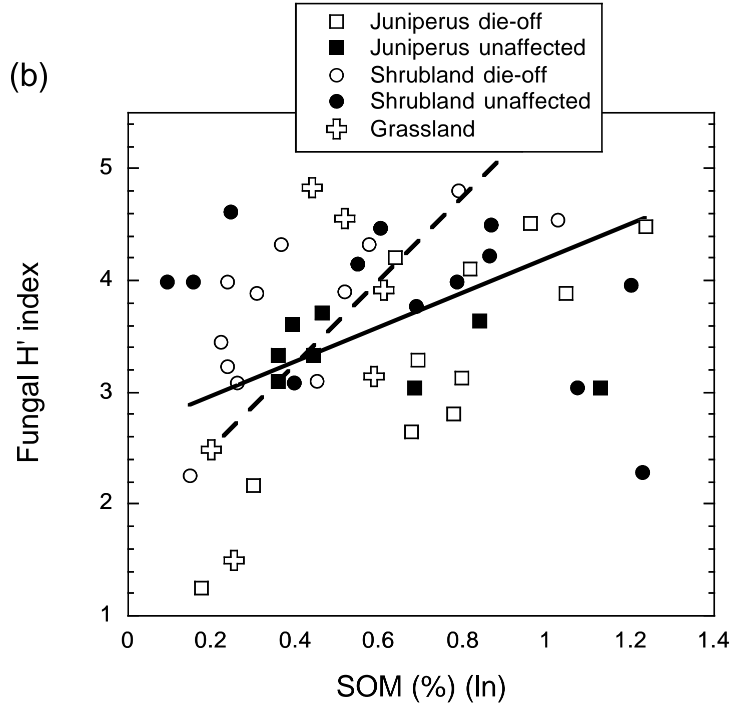
817

818

819



820



821
822
823 Figure 4
824

825 Figure 4. Relationship between fungal diversity and SOM (%): (a) fungal richness
826 (number of TRF peaks) and (b) fungal H' index, estimated from the area of TRF peaks,
827 and SOM in the five studied habitats. Linear fit for unaffected stands (solid line) (richness,
828 r^2=0.08, p = 0.210; H', r^2=0.09, p = 0.193) and grassland (dashed line) (richness, r^2=0.75,
829 p = 0.012; H', r^2=0.56, p = 0.053) is shown in (a) and (b) to illustrate the results of GLM
830 models (see text).

831

Table 1. Mean (SE) vegetation and soil properties in the different vegetation types and climate-induced die-off stands. The number of final samples used in the analyses of bacterial and fungal communities is provided in the last two columns. Letters indicate significant differences (one-way ANOVA and pair-wise t test). Soil variables were log transformed to attain normality. Defoliated canopy: percentage of defoliation in relation to total canopy cover, estimated in woody stands; H': Shannon diversity index; SOM: Soil Organic Matter, BR: Basal Soil Respiration, SIR: Substrate-induced Soil Respiration, MBC: Microbial Biomass Carbon, qCO_2: Microbial Metabolic Quotient.

	Defoliated canopy (%)	Vegetation Richness	Vegetation H'	pH	Conductivity (µS/cm)	SOM (%)	BR (mg CO2/kg·h)	SIR (mg CO2/kg·h)	MBC (µg C/g)	qCO_2	Bacteria samples	Fungi samples
Unaffected Juniperus woodland	0.83 a (0.61)	5.69 a (0.60)	0.53 c (0.08)	6.50 a (0.08)	159.7 a (16.1)	2.06 a (0.15)	6. ab (1.05)	10.1 a (1.2)	74.6 a (15.3)	0.121 ab (0.029)	11	8
Die-off Juniperus woodland	82.7 b (2.89)	8.23 b (0.91)	1.55 a (0.09)	6.41 a (0.07)	143.7 a (13.9)	2.22 a (0.16)	8.39 bc (1.11)	18.5 bc (1.9)	72.7 a (10.1)	0.131 ab (0.030)	14	11
Unaffected shrubland	6.6 a (3.18)	8.46 b (1.06)	1.19 b (0.10)	6.33 a (0.08)	115.4 ab (18.0)	2.09 a (0.22)	4.73 a (1.06)	9.7 a (1.0)	63.4 a (11.6)	0.101 a (0.111)	11	13
Die-off shrubland	79.2 b (3.18)	9.0 b (0.75)	1.67 a (0.13)	6.48 a (0.10)	105.6 ab (7.9)	1.60 b (0.12)	8.57 bc (0.94)	12.7 ab (0.9)	68.7 a (12.9)	0.262 b (0.470)	10	12
Grassland	-	9.5 b (0.61)	1.59 a (0.07)	6.38 a (0.11)	87.7 b (11.9)	1.86 ab (0.16)	13.06 c (3.34)	23.2 c (4.5)	78.4 a (14.7)	0.195 ab (0.180)	13	8

839
840 Table 2. Models analyzing bacterial diversity in relation to climate-induced die-off (Die-off model).
841 These models were only applied in *Juniperus* woodlands and shrubland. Only factors contributing
842 to the final, selected model are shown; other factors did not show statistical significance. Results
843 from full models and models excluding vegetation diversity are shown (see Methods). Categorical
844 variables were coded hierarchically after splitting the levels in two groups, as indicated by
845 brackets (see Methods). H': Shannon diversity index; SOM: Soil Organic Matter; R: Raposo; M:
846 Marques; O: Ojillo; J: *Juniperus* woodland, S. Shrubland.
847
848

full model			**Die-off model**			
	Richness		Evenness		H'	
AIC	444.59		-186.09		15.87	
p	<0.001		<0.001		0.058	
r^2	0.279		0.248		0.079	
Model terms	est	p	est	p	est	p
Locality ([R,M],O)	-	-	-	-	p=0.058	p=0.058
SOM	-30.72	0.032	-	-	-	-
Vegetation diversity	25.85	0.002	-0.031	<0.001	-	-
without Vegetation Diversity						
AIC	448.32		-182.86		15.29	
p	0.005		0.003		0.058	
r^2	0.218		0.275		0.079	
Model terms	est	p	est	p	est	p
Locality (R,[M,O])	-	-	-0.009	0.083	p=0.058	p=0.058
Vegetation (J,S)	-	-	0.009	0.048	-	-
Die-off	11.78	0.010	-0.014	0.004	-	-
SOM	-30.75	0.039	-	-	-	-

849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865

866 Table 3. Models analyzing fungal diversity in relation to climate-induced die-off (only for *Juniperus*
867 woodlands and shrubland) (Die-off model). Categorical variables were coded hierarchically after
868 splitting the levels in two groups, as indicated by brackets (see Methods). Only factors
869 contributing to the selected final model are shown; other factors did not show statistical
870 significance. H': Shannon diversity index; SOM: Soil Organic Matter; J: *Juniperus* woodland, S.
871 Shrubland.
872

	Die-off model					
	Richness		Evenness		H'	
AIC	102.05		-52.19		84.09	
p	0.015		<0.001		<0.001	
r^2	0.300		0.393		0.469	
Model terms	est	p	est	p	est	p
Vegetation (J,S)	0.004	0.971	-0.069	<0.001	-0.383	0.002
Die-off	-0.108	0.323	0.006	0.722	0.012	0.895
SOM	0.339	0.323	0.109	0.067	0.68	0.019
Die-off x SOM	0.937	0.010			1.35	<0.001
Vegetation (J,S) x Die-off	-0.356	0.003	0.048	0.015	-	-

873

Table 4. Models analyzing soil respiration in stands of *Juniperus* woodlands,and shrubland subjected to climate-induced die-off. Different models were built for the three indicators of diversity: richness, evenness and H'. Categorical variables were coded hierarchically after splitting the levels in two groups, as indicated by brackets (see Methods). Only factors contributing to the selected final model are shown; other factors did not show statistical significance. For a given respiration variable, when microbial diversity was not significant, the models converged to the same result and we use a common column heading. Rich: richness; Even: evenness, H': Shannon diversity index; SOM: Soil Organic Matter; R: Raposo; M: Marques; O: Ojillo.

	Substrate-induced Soil Rspiration (SIR)						**Basal Soil Respiration (BR)**						**Microbial Metabolic Quotient (qCO_2)**					
	Rich		Even		H'		Rich		Even		H'		Rich		Even		H'	
AIC	27.9		35.3		29.6		79.5		80.8		64.6		67.6		66.1		67.7	
r^2	0.603		0.591		0.564		0.444		0.429		0.423		0.622		0.650		0.614	
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Model terms	est	p	est	p	est	p	est	p	est	p	est	p	est	p	est	p	est	p
Locality (R,[M,O]	-0.150	0.007	-0.173	0.007	-0.178	0.004	-0.152	0.093	-	-	-	-	-0.147	0.133	-	-	-	-
Locality (R)	-	-	-	-	-	-	-	-	-0.331	0.011	-0.305	0.019	-	-	-0.272	0.056	0.289	0.04
Locality (M)	-	-	-	-	-	-	-	-	-0.006	0.747	-0.036	0.954	-	-	-0.065	0.578	0.044	0.716
Die-off	0.323	<0.001	0.281	<0.001	0.269	<0.001	0.449	<0.001	0.417	<0.001	0.39	<0.001	0.461	<0.001	0.392	<0.001	0.393	<0.001
Vegetation	0.062	0.196	0.091	0.107	0.102	0.005	-	-	-0.022	0.791	-	-	-	-	-	-	-	-
Bacterial diversity	-0.006	<0.001	0.853	0.615	-0.580	0.004	-0.008	0.007	3.040	0.252	-0.720	0.025	-0.008	0.009	2.540	0.353	0.700	0.037
Microbial biomass C	-	-	-0.145	0.081	-	-	-	-	-	-	-	-	-1.030	<0.001	-1.010	<0.001	1.020	<0.001
Die-off x Bacterial diversity	-	-	-3.65	0.046	-	-	-	-	-7.180	0.007	-	-	-	-	-	-	-	-
Vegetation x Bacterial diversity	-	-	4.32	0.018	-	-	-	-	5.11	0.036	-	-	-	-	-7.490	0.010	-	-
Vegetation x Die-off	0.157	0.003	0.219	<0.001	0.154	0.005	-	-	-	-	-	-	-	-	-	-	-	-

881