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1 **Resilience of soil microbial communities impacted by severe drought and high**
2 **temperature in the context of Mediterranean heat-waves**

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15 **Abstract**

16 In the context of Climate Change, the increasing of frequency and intensity of droughts and
17 heat-waves constitutes a serious threat for agroecosystems in the Mediterranean region. Soils
18 and their functions may be impacted by these extreme events through changes in the biomass,
19 composition and activities of edaphic microbial communities. We designed an experiment to
20 investigate changes over time in the microbial biomass, composition (EL FAME profiles) and
21 functions (catabolic responses) after severe drought and high temperature disturbances.
22 Impacts were assessed using indoor soil microcosms under controlled drought and high
23 temperatures, mimicking various stress scenarios and durations in conditions of severe

24 drought and heat wave. Drought and heat wave restructured the soil microbial communities
25 over the course of the experiment. This may be a consequence of inhibition and/or killing of
26 sensitive species and selection of tolerant species by the disturbances applied, but also of the
27 proliferation of fast growing species after environmental soil conditions had been restored.
28 Heating dry soil at 50°C had a stronger effect than only drying. Moreover, above a critical
29 threshold of heat wave duration, soil microbial communities may have undergone a drastic
30 biomass killing and restructuring associated with a shift in physiological traits. In this
31 experimental context, resilience of microbial catabolic functions was not observed and in
32 consequence ecosystem processes such as carbon mineralization and sequestration in soil may
33 be affected.

34 **Key words:** microbial community structure, soil, drought, heat-wave, substrate-induced
35 respiration, resilience

36 1. Introduction

37 Global warming circulation models predict changes in spatial and temporal patterns of
38 precipitation, including shifts in the frequency and intensity of droughts and heat-waves [1,2].
39 This is especially true in the Mediterranean region [3]. In August 2003, a large part of
40 Western Europe, including France, was affected by a heat wave of exceptional duration,
41 intensity and geographical extent. Its impacts were drastic, including the death of thousands
42 of people, and considerable effects on ecosystems and agricultural production [4]. Extreme
43 climatic events may be more important drivers of ecosystem functioning than mean
44 conditions [5,4]. Soils and their functions may be impacted by these events through changes
45 in soil temperature, water content and nutrient dynamics [6].
46 Soil microbial communities are highly diverse in their composition and play an essential role
47 in nutrient cycling functions such as organic matter decomposition and mineralization,

48 nutrient mobilization [7]. Recent studies suggested the importance of microbial diversity for
49 soil functioning, and if the processes that determine the rate, at which microbial services are
50 ensured, are starting to be identified, changes in microbial communities due to environmental
51 factors (i.e. temporal shifts in temperature and soil moisture), and their link with soil
52 metabolic functioning should be more investigated [8]. In particular, the impact of stresses or
53 disturbances on soil resilience (i.e. recovery over time after a stress in comparison to control)
54 are of particular importance, in order to understand how compositional shifts changes may
55 affect soil processes and thus ecosystem services [9].

56 Several studies have shown the separate impacts of soil moisture and climate warming on
57 biogeochemical cycles, and on the composition and activities of microbial communities
58 [10,11,12]. However, the combined effects of changes in temperature and moisture have been
59 less investigated [13], in particular, to evidence the impact of extreme events such as severe
60 droughts and heat waves in temperate soil ecosystems [14], in relation with their intensity and
61 duration. If these disturbances exceed a critical threshold the community may exhibit a
62 dramatic change in composition that would prevent activities from fully recovering [15]. In
63 consequence, soil processes such as organic matter decomposition and nutrient cycling may
64 be durably affected resulting in a loss of ecosystem functions and services [4].

65 There is a need to investigate more systematically the long-term impact of severe drought and
66 heat wave on the soil microbial community, particularly in Mediterranean soils as they may
67 be more affected. The aims of this study were to assess both short-term responses and
68 changes over time of soil microbial biomass, composition and catabolic functions to various
69 disturbances. These disturbances were studied using soil microcosms under controlled
70 drought and high temperatures, mimicking various scenarios and durations in conditions of
71 severe drought and heat wave. We hypothesised (1) that the effects of drying-heating on soil
72 microbial communities may be greater than the effects of drying alone, and (2) that the

73 disturbance duration may increase shifts in microbial taxonomic structure decreasing recovery
74 of catabolic functions and carbon allocation.

75 **2. Materials and methods**

76 **2.1. Soil**

77 The soil selected for this study was a Mediterranean agricultural cambisol. The soil-sampling
78 site was a conventionally farmed pea field (INRA-Avignon, South Eastern France, 43° 54' 57"
79 N and 4° 52' 58" E) and had received no chemical treatment in the month preceding soil
80 sampling. Soil was sampled (25/03/08) from the 0-20 cm depth, with 20 soil cores (5 cm
81 diameter) taken at random around the field and pooled (14.9% gravimetric water content).
82 Following collection, visible soil organisms, roots and plant debris were removed and soil
83 sample was air-dried. After drying (duration less than 24 hours), the soil was sieved to obtain
84 homogenized 2-3 mm-sized aggregates (6.6% gravimetric water content).
85 The soil is a fine calcareous silty clay loam with a texture of 326 g kg⁻¹ clay, 270 g kg⁻¹ silt
86 and 33 g kg⁻¹ sand. The soil aggregates contained 11.6 g organic C kg⁻¹ soil, 1.42 g total N kg⁻¹
87 soil and 365 g CaCO₃kg⁻¹ soil, and had a pH (water) value of 8.37. Water holding capacity
88 (WHC = 31.4%) of the soil aggregates was measured as the gravimetric water content of 10 g
89 of soil fully saturated with water and then left to stand for half an hour in a filter funnel filled
90 with glass wool.

91 **2.2. Experimental design**

92 Before the experiment, soil aggregates were slowly capillary-wetted and maintained at -0.01
93 MPa (21.4 % water content) on a suction table for 2 weeks at ambient temperature (25°C +/-
94 2) and in the dark, to ensure uniform initial conditions.

95 The soil was divided (50 g of aggregates) between 62 Petri boxes (9 cm diameter
96 microcosms, partitioned in 14 controls and 8 microcosms per treatment). The following

97 treatments were then performed: (1) Control (C) soil incubated at constant conditions (-0.01
98 MPa, 25°C), (2) soil subjected to drying disturbance (D) (-10 MPa, 4.5 % water content) and
99 incubated at 25°C (mimicking a severe drought alone), (3) soil subjected to drying-heating
100 disturbance (D-H) (-10 MPa, 4.5 % water content) and incubated at 50°C (mimicking a severe
101 drought combined to an heat wave). All treatments were conducted in darkness and at a
102 constant temperature, with each microcosm placed in a dessicator inside an incubator. The
103 dessicators were opened every 3 days to ensure aerobic conditions and soil water contents in
104 the microcosms were regularly checked by gravimetric measurements (if necessary, water
105 content was adjusted by gently spraying water into microcosms).

106 The total duration of the experiment from application of the stress was 84 days.

107 The experimental conditions were applied as follows (Fig. 1):

108 Microcosms of control soils (C) previously equilibrated to -0.01 MPa water potential were
109 kept at 25°C +/-1 in a control-dessicator with a beaker containing free water to maintain high
110 air humidity, throughout the course of the experiment (84 days). For the drying and drying-
111 heating treatments, the soils in the microcosms were rapidly adjusted to water content close to
112 the target water potential of -10 MPa. This was done by increasing airflow over a silica
113 desiccant, at a temperature of 25°C. The microcosms were then divided between two drought-
114 dessicators. The bottom of each drought-dessicator contained a saturated aqueous solution of
115 potassium nitrate salt that controlled the air humidity, a water potential of -10 MPa was
116 maintain in the soil microcosms [16]. One drought-dessicator was then placed in an incubator
117 at 25°C +/-1 (treatment D) and the other in an incubator at 50°C +/-1 (treatment D-H). Three
118 durations of treatment were then chosen: (1) Two days, which is the time scale of most
119 drying-rewetting experiments (e.g. [17]), (2) 7 days, which corresponds to a “heat wave” for
120 climatologists, (3) 21 days, which was the actual duration of the 2003 heat wave in the area of
121 the sampled field (Avignon, France). After each treatment duration, treated soils were

122 rewetted and placed under the same conditions as those of the controls (-0.01 MPa, 25°C)
123 until the end of the experiment.

124 The short-term impacts (resistance) and long term ones (resilience) after restoration of the
125 microbial parameters in the treated soils were compared to the controls [9]. Resistance of
126 microbial communities was evaluated 2 days after the end of disturbance. Several studies
127 showed that resilience varies according to the type and duration of stress applied (e.g.
128 [18,19]), and in the case of drying and rewetting stress, Fierer and Schimel [20] observed no
129 functional resilience 6 weeks after the end of stress. We then have chosen experiment
130 duration of 84 days (corresponding to 63 days after the end of the longest disturbance
131 applied). Resilience of microbial communities was finally evaluated on the day corresponding
132 to 3 times the disturbance duration after end of disturbance (i.e. 6-day resilience for 2-day
133 treatments, which means sampling at day 8 of experiment; 21-day resilience for 7-day
134 treatments, sampling at day 28 of experiment; and 63-day resilience for 21-days treatments,
135 sampling at day 84 of experiment). Soils were sampled and analyzed just before and after
136 restoration of control conditions, and at different dates until the end of the experiment: 6 to 8
137 g of moist soil were collected in four randomly selected microcosms from each treatment
138 scenario (the remaining soil of the microcosm was further incubated, after weighting).

139 Dissolved organic carbon (DOC) analysis and substrate-induced respiration (SIR) giving
140 community-level physiological profiling (CLPP) were performed immediately after sampling;
141 fatty acid (EL-FAME) extractions and analysis were performed subsequently on deep-frozen
142 soils (-25°C).

143 To confirm the results obtained from our experiment, it was repeated on the same soil with
144 the higher disturbance D-H-21, compared to control. DOC and CLPP measurements were
145 then performed at two sampling dates (23 and 84 days from start of stress) for this repeat
146 experiment.

147 **2.3. Soil chemical and microbial properties**

148 2.3.1. Total dissolved organic C (DOC)

149 Two g of the fresh soil were extracted with 10mL of 0.5M K₂SO₄ for one hour on a shaker.

150 K₂SO₄ extracts were filtered through 0.45 mm filters and analyzed for extractable C

151 (Shimadzu TOC-5050A total organic C analyzer).

152 2.3.2 Soil respiration and Community Level Physiological Profiles (CLPP) on substrate- 153 induced respiration

154 Basal (BR) and substrate-induced soil respiration (SIR) were assessed using the MicroRespTM
155 system of Campbell et al. [21], consisting of a 96-deep-well microplate (Nunc 278012 1.2mL

156 volume) filled with soil and addition of water only (BR) or aqueous carbon substrates (SIR),

157 sealed individually to a colorimetric CO₂-trap microplate. Mineralization of 11 carbon

158 substrates was tested for CLPP: four carbohydrates (glucose, sucrose, trehalose and

159 cellulose); 3 amino acids (glycine, alanine and arginine); 3 carboxylic acids (malic acid, and 2

160 phenolic acids: caffeic acid and ellagic acid) and a phenol (catechol). The carbon substrates

161 were selected for ecological relevance: Relevant to soil agro-ecosystems (i.e. plant residues,

162 root exudates etc.), involved in desiccation tolerance (osmolytes like sucrose, trehalose and

163 glycine [14]), and differentially mineralized. The carbon substrates were prepared in order to

164 add 30 mg of C per mL of soil water into each deep well. Substrates that did not readily

165 dissolve in water (caffeic acid and ellagic acid) were supplied at a concentration of 15 mg C

166 mL⁻¹ soil water. Stock solutions of the carbon substrates were adjusted to soil pH (pH 8.4)

167 using NaOH or HCl to avoid any substrate-pH effects on microbial communities and to

168 minimize chemical artefacts due to carbonate-derived CO₂. The concentration of the reactants

169 in the Cresol Red gel detector CO₂-trap microplate was the same as described by Campbell et

170 al. [21]. Each deep-well microplate was sealed to the CO₂-trap microplate with a silicone joint

171 (MicroRespTM, UK) and incubated in the dark at 25°C. CO₂-trap absorbance was measured at

172 590 nm with a spectrophotometer (HTS 7000 de Perkin Elmer) immediately before to sealing
173 to the microplates, and after 6 h incubation. A calibration curve of absorbance versus
174 headspace equilibrium CO₂ concentration (measured by gas chromatography) was fitted to a
175 regression model.

176 The metabolic quotient qCO₂ is the ratio of basal respiration (BR) to microbial biomass, and
177 is a sensitive ecophysiological indicator of soil stress induced by environmental conditions
178 [22]. Microbial biomass was determined by glucose-induced respiration (GIR) [23] with
179 MicroResp™. We divided BR by GIR to obtain an index directly correlated to qCO₂ but unit-
180 less.

181 2.3.3. Microbial community structure (EL-FAMES)

182 We used microbial fatty acids to determine microbial structure. Fatty acid profiling provides
183 information on soil microbial biomass and community structure because phospholipids
184 (constituents of the cell membranes) are rapidly degraded after cell death and their relative
185 abundance differs between specific groups of microorganisms [24]. We performed the ester-
186 linked fatty acid methyl esters (EL-FAMES) method modified from [25] Schutter and Dick
187 2000. Three g of soil (three replicates) were extracted with 15 ml of 0.2 M KOH in methanol
188 (37°C, agitation, one hour). The pH of this buffer was neutralized with 2.65 ml of 1.0 M
189 acetic acid. The EL-FAMES were extracted by adding 10 ml hexane, which was removed (5
190 ml) from the aqueous phase after centrifugation at 800xg for 20 min. The hexane was
191 evaporated under a stream of N₂. EL-FAMES were resuspended in 170 µl hexane with an 30
192 µl internal standard (methyl stearate 0.01 M, 18:0) and analyzed by gas chromatography (GC)
193 with an (GC Agilent 7890, USA). The GC capillary column was a medium polar cyanopropyl
194 column (DB 23) 60 m long, with an internal diameter of 0.25mm and film thickness of
195 0.15µm. Flame ionization detection (FID) was performed at a temperature of 280°C using
196 helium as a carrier gas at a flow rate of 30 ml min⁻¹. Identification of the FAMES was

197 performed automatically using the Agilent Chemstation software (Agilent, Inc., Palo Alto,
198 CA) in combination with the Agilent Retention Time Locking (RTL) library for FAMES. The
199 FAME database was extended with a mixture of 24 microbial FAMES (Bacterial Acid Methyl
200 Esters Mix 47080-U; Supelco, Inc.) and 10-Me16:0 (Matreya). Biomarkers of specific
201 functional groups were assigned according to classification of Frostegård et al. [25]. Iso- and
202 anteiso-fatty acids were taken as Gram-positive (G+) bacterial biomarkers (i15:0, a15:0,
203 i16:0, i17:0), and mono-unsaturated and cyclopropyl fatty acids (16:1 ω 7c, 17:0 cy, 18:1 ω 7c,
204 19:0 cy) as Gram negative (G-) bacterial biomarkers [26]. The FAMES 18:2 ω 6c and
205 10Me16:0 were used as indicators for the fungi (FUNG) and actinobacteria (ACT)
206 populations respectively [26,24]. The sum of all FAMES (nmol g⁻¹ dry soil) was used as an
207 estimate of microbial biomass. The ratio of saturated to unsaturated fatty acids
208 (SAT/UNSAT) was used as a stress indicator [27]. FAMES that occurred as less than 5% of
209 all samples were excluded from the data set.

210 **2.4. Data analysis**

211 The values of the microbial parameters (BR, GIR, qCO₂, EL-FAME biomarkers) and DOC
212 for the different treated soils were normalized to the control values % change from control
213 [28]. To compare treatments and disturbance duration impacts on microbial parameters and
214 DOC we applied non-parametric statistical procedures ([29]. Mann-Whitney tests were
215 performed on raw data to assess the difference between the control and treated microcosms;
216 Kruskal-Wallis tests and post hoc paired comparisons tests (Bonferroni) were performed on
217 % change from control to assess the difference between disturbance durations (n = 4 for SIR
218 measurements, BR and qCO₂, n = 3 for DOC and EL-FAMES measurements, the significance
219 level was set to 5%). Non-parametric redundancy discriminant analysis (RDA), followed by
220 a Monte Carlo permutation test (5000 random permutations; CANOCO 4.5 software; [30])
221 were used to test the statistical significance of the explanatory variables (factors): disturbance

222 treatment-type, disturbance duration and recovery time (i.e. time elapsed between end of
223 disturbance and time of measurement), and the interactions between these factors on CLPPs
224 and EL-FAME data. The EL-FAMEs structural differences and CLPP functional community
225 differences between treated and control soils were described using the Bray-Curtis
226 dissimilarity index (BCI, [31]).

227 3. Results

228 Mean values of biotic parameters and DOC of control soil are listed in the Table 1.

229 3.1. Soil respiration, metabolic $q\text{CO}_2$ and DOC

230 Soil-microcosms subjected to disturbance lasting 21 days, whether drying (D) or drying-
231 heating disturbance (D-H), showed changes in basal respiration (BR). Increased respiration
232 was detected 2 days after the end of the disturbance (Tab.2), with a maximum value of 0.88
233 $\mu\text{g CO}_2\text{-C dw.g}^{-1} \text{h}^{-1}$ for the D-H-21 disturbance treatment (compared to 0.25 $\mu\text{g CO}_2\text{-C dw.g}^{-1}$
234 h^{-1} for the control at day 23, Tab.1). Basal respiration recovered to control values at
235 resilience-dates (0.60 $\mu\text{g CO}_2\text{-C dw.g}^{-1} \text{h}^{-1}$ at day 84, Tab.1) for soil-microcosms subjected to
236 drying. For soil-microcosms subjected to a drying-heating disturbance no resilience was
237 apparent at the end of experiment and D-H soil-microcosms showed a decrease in BR
238 compared to the control (Mann-Whitney tests; $p \leq 0.05$, $n=4$) (Tab.2).

239 Except for the 7 days disturbance duration, disturbed soil showed a significant increase in
240 metabolic $q\text{CO}_2$ two days after the end of the disturbance (Mann-Whitney test; $p \leq 0.05$; $n=4$)
241 (Fig. 2. A-B), especially for the 21 days drying-heating disturbance (D-H-21). After that,
242 metabolic $q\text{CO}_2$ decreased to control values over the course of the experiment, but remained
243 significantly higher for soil subjected to D-H-21 disturbance treatment (Mann-Whitney tests;
244 $p \leq 0.05$; $n=4$). DOC presented high concentrations in soil two days after the end of

245 disturbance (with a maximum of $136.7 \mu\text{g C dw.g}^{-1}$) and no recovery for D-H-21 disturbance
246 treatment (Mann-Whitney tests; $p \leq 0.05$; $n=3$) (Fig. 2. C-D).

247 Except for the resilience in $q\text{CO}_2$ and DOC parameters of drying-disturbed soils, disturbance
248 durations (2, 7 and 21 days) have significant effects on % change relative to control of BR,
249 $q\text{CO}_2$ (Kruskal-Wallis tests; $p \leq 0.05$; $n=4$) and DOC (Kruskal-Wallis tests; $p \leq 0.05$; $n=3$) in
250 term of both resistance and resilience (Tab.2, Fig.2). Generally, the longer the disturbance
251 duration, the greater the impact (especially with high-temperature disturbance on Basal
252 Respiration, Post hoc paired comparisons test $p \leq 0.05$). However, this pattern was not
253 straightforward. In some cases, 2-day disturbances had a stronger impact than 7-day
254 disturbances (i.e. $q\text{CO}_2$ of drying-heating-disturbed soils, Post hoc paired comparisons test
255 $p \leq 0.05$) (Fig.2).

256 3.2. Community Level Physiological Profiles (CLPPs)

257 The redundancy analyses (RDA) performed on the MicroRespTM profiles (CLPPs) showed
258 that disturbance treatments (D and D-H), disturbance durations (2, 7 and 21 days) and post-
259 disturbance recovery time, all had significant effects on the catabolic functions of the soil
260 microbial communities (Fig. 3). Moreover, Figure 3-A shows that disturbance duration
261 interacts significantly with the type of disturbance treatment: the longer the disturbance
262 duration the greater the changes in CLPPs, especially for dried-heated soils (D-H). Figure 3-B
263 shows that the differences between CLPPs in disturbed soils increase with post-disturbance
264 recovery time: Controls (C) differ sharply from the dried-heated soils (D-H), with the dried
265 soils (D) occupying an intermediate position on the factorial map. In terms of resistance and
266 resilience, the Bray and Curtis dissimilarity indices (BCI) confirm the RDA analyses. The
267 effects of disturbance duration on CLPP using BCI index were significant (Kruskal-Wallis
268 tests, $p \leq 0.05$, $n=4$), and the effects of disturbance treatment type were also significant for the
269 7-day and 21-day disturbances (Tab.2, Mann-Whitney tests, $p \leq 0.05$, $n=4$).

270 Soil respiration on osmolyte substrates (such as sucrose, trehalose and glycine) were not
271 differentially affected by the disturbances, compared to other substrate-induced respirations
272 (data not shown).

273 **3.3. Microbial community structure (ELFAMEs)**

274 The redundancy analysis (RDA) performed on the EL-FAMEs shows that disturbance
275 treatment interacts significantly with disturbance duration (7 and 21 days) (Fig 4-A): the
276 disturbance duration effect was particularly strong with the soils subjected to D-H
277 disturbance. Figure 4-B- shows that disturbance treatment also interacts significantly with
278 post-disturbance recovery time. And this was confirmed by the BCI performed on EL-
279 FAMEs: the effects of the disturbance treatments on EL-FAMEs using BCI index were only
280 significant for the 21-day disturbances (Tab. 2, Mann-Whitney tests, $p \leq 0.05$, $n=3$).
281 The effects of D disturbances on the EL-FAME biomarkers differed from the effects of D-H
282 disturbances. The difference was particularly marked for the longest disturbance duration (21
283 days, Fig. 5). More precisely, the D-21 disturbance, just before rewetting the soil, had no
284 significant effect on the EL-FAME biomarkers (Fig. 5-A) except for G+ indicators, which
285 increased compared to the control (18.1% +/- 3.9). The short-term impact of drying/rewetting
286 (two days after rewetting at end of disturbance) was a decrease in EL-FAME biomarkers (Fig.
287 6-A), except for G+ indicators. Almost all EL-FAME biomarkers of D-21 soil had returned to
288 the level of the control soil by day 22 after the end of the drying disturbance and remained at
289 that level until the end of the experiment. The one exception was ACT, which decreased (-
290 20.0% of control +/- 4.7, day 84). The D-H-21 disturbance, just before rewetting of the soil,
291 had various effects on the different EL-FAME biomarkers (Fig. 5-B). At this stage, there was
292 a strong decrease for FUNG (-72.8% +/- 26.5) and an increase for G+ indicators (17.3% +/-
293 2.9) compared to the control. Two days after rewetting and lowering the temperature, the
294 short-term impact of the D-H disturbance was a stronger decrease of G+ indicators and ACT,

295 and a weaker one for G- indicators and FUNG. All EL-FAME biomarkers in the D-H-21 soil
296 were lower than the control values. Over the course of the experiment some inhibited
297 microbial groups were stable (G- and FUNG) while others showed increased inhibition (G+
298 and ACT). At day 84, ACT had completely disappeared (n = 3). No EL-FAME biomarkers in
299 the D-H-21 soil remained at the same level as in the control soil.

300 The saturated/unsaturated fatty acids ratio was raised by drought and heat wave disturbances
301 (Table 2), especially for the 21-day disturbances (at day 63 the SAT/UNSAT ratio of the D-
302 H-21 soils was still high: 20.42). At the end of experiment the collapse (with a high
303 variability) of the SAT/UNSAT ratio of the D-H-21 soil was due to the collapse of all the
304 microbial biomass at this date (confirmed by total EL-FAMES and SIR, Tab. 2).

305 **4. Discussion**

306 **4.1. Consequences of severe drought and high temperature on both short and long-term** 307 **responses of soil microbial communities**

308 The stimulation of the basal respiration two days after end of disturbance suggests an increase
309 in C substrate availability, which is often a limiting factor for both microbial growth and
310 activities, regardless the disturbance regime. The concomitant increase in DOC amounts
311 confirms this hypothesis. This process called “Birch-effect” [32] can be attributed to two
312 mechanisms [33]. One is the “substrate supply” mechanism; a physical disruption of soil
313 aggregates due to drying-rewetting cycle (and probably also heat; [34]), which releases soil
314 organic matter making it available to microorganisms [35,17]. The other is the “microbial
315 stress” mechanism; releases of two sources of microbial carbon after disturbance: (i) from
316 dead and lysed microbes due to drought, heat and rewetting [28], and (ii) from osmolyte
317 intracellular compounds synthesized during drying stress [17]. DOC from lysed microbes is
318 confirmed by an important decrease in microbial biomass (SIR Glucose and total EL-FAMES)

319 for most of disturbance regimes. We were not able to show via our MicroRespTM
320 measurements on specific C substrates, if some drought-disturbed microbial communities
321 would be specifically adapted to mineralizing osmolyte substrates. Therefore in our case, the
322 microbial DOC mineralized after the disturbance may result mainly from cell death instead of
323 osmolyte release [32]. For the 2 and 7 days disturbance durations, the “Birch-effect” showed
324 two days after rewetting was slight, probably because of a beginning of resilience of the
325 pulses of CO₂ and DOC after rewetting [17] in this Mediterranean soil adapted to frequent
326 wet/dry cycles.

327 At long term, a 21 days duration heat wave, in contrast to drought, dramatically impacted
328 microbial biomass (total EL-FAMEs amounts and SIR) suggesting a lasting overall impact
329 [36]. As we have seen, metabolic qCO₂ recovered to control values after the disturbance in
330 drought soils but remained high until the end of the experiment in drought-heated soils. At
331 short-term, the high values of this BR to biomass ratio are a consequence of direct killing
332 biomass induced by both disturbance regimes. The qCO₂ is also known to increase when
333 microbial communities divert a higher proportion of carbon source to maintenance-energy
334 requirements than to biosynthesis as a result of an exogenous disturbance [22]. The
335 physiological costs imposed on soil microorganisms [17], as well as direct killing of
336 microbial biomass especially by heat wave disturbances, may be great enough to cause at long
337 term shifts in the allocation and fate of carbon in the entire microbial community.

338 EL-FAME community structure measurements differentiated between the impacts of drought
339 and heat wave on the composition of the microbial community, with an intermediate effect
340 and resilience for soils subjected to drought, in contrast to soils subjected to heat wave, which
341 did not present resilience. Moreover, changes in CLPP also suggest differences of impact in
342 functional structure, concomitant to taxonomic shifts.

343 The decrease in the concentration of microbial EL-FAME biomarkers in treated D-21 soil two

344 days after restoration reveals the impact of an osmotic shock of re-wetting induced by
345 rewetting rather than a direct drying disturbance impact [12]. The exception of G+ organisms,
346 which were not inhibited by drying or by rewetting, may be explained by their thicker cell
347 walls, giving capacities of adaptation to changes in water potential [37]. Fungi, as well as
348 Actinobacteria, are known to be tolerant to drying [37], but in our study they were affected by
349 drying-rewetting because of their sensitivity to osmotic shock of rewetting [38,12].

350 In the context of heat wave disturbance the restoration of environmental soil conditions
351 (rewetting and lowering temperature) also impacted drastically on microbial community
352 composition. G+ biomarkers were not impacted by the combination of high temperature and
353 drought (as shown at day 21). G+ i17 :0, which was the major EL-FAME biomarker of the
354 G+ group, indicates a bacterial group known to be thermophilic [27]. However, in contrast to
355 their behaviour in the dried-rewetted microcosms, in D-H microcosms the G+ group
356 presented sensitivity to rewetting and lowering temperature (from day 21 to day 23).

357 Actinobacteria (known to be sensitive to high temperatures; [27]) were dramatically impacted
358 by heat wave. The 50°C temperature disturbance combined with the osmotic shock of re-
359 wetting probably impacted irreversibly on the biomass of this known K-strategist group.

360 Fungi (also known to be sensitive to high temperatures; [27]) were strongly impacted by heat
361 wave disturbance; if the restoration of environmental soil conditions did not induce further
362 inhibition of this microbial eukaryote group, we did not measure any resilience. In a recent
363 study on recovery of soil microbial community subjected to high temperatures, Barcela-
364 Moreno and Bååth [39] showed that heating of soil shifted the recolonizing microbial
365 community from fungi towards bacteria. The G- EL-FAME biomarkers were impacted by
366 heat wave disturbance but stayed at the same concentration levels throughout the experiment
367 after restoration; they were thus the least inhibited group at the end of the experiment. Gram-
368 negative bacteria like Proteobacteria are r-strategists (or copiotrophic, [40]) and their ability

369 to grow fast may have favoured their biomass stability, or even growth, as shown in the D-21-
370 treated soil at the end of experiment. Similarly, Thompson et al. [41] observed high CO₂-C
371 efflux correlated with the development of Proteobacteria in soils previously dried and
372 rewetted.

373 The increases in ratio of saturated to unsaturated fatty acids we measured in disturbed soils
374 may be attributed to rapid physiological adjustments by certain microorganisms and
375 especially to higher survival rates in drought and high temperature tolerant species compared
376 to others [27].

377 However, one must bear in mind that the EL-FAME extraction method presents some
378 limitations for characterizing microbial communities in environmental samples
379 (contaminations with no microbial FAMES, [24]). And because of possible conservation of
380 FAMES on dead microbial cells in dry environmental conditions, this may have led us to
381 underestimate the impact of desiccation on the microorganisms [28].

382 To sum up, the restructuring of microbial communities during the course of the experiment
383 after the end of heat wave disturbance may be a consequence of drastic impact on K strategic
384 sensitive species (i.e. Fungi and Actinobacteria), selection of tolerant species (i.e. G +
385 bacteria), and the development of fast-growing copiotrophic species (i.e. G - bacteria) after
386 the restoration of environmental soil conditions, stimulated by soil organic matter that had
387 become available from killing biomass and lack of competition.

388 Figure 6 presents a schematic representation of the soil microbial taxonomic and functional
389 responses to the heat wave (Fig. 6A) and drought (Fig. 6B) 21-day disturbances in the context
390 of our experiment. Drought and rewetting is characterized by a “Birch effect” and resilience
391 occurring about 30 days after the end of disturbance. This recovery suggests the low impact
392 of a severe drought on the physiological traits of this Mediterranean soil, which *in situ* was
393 frequently exposed to moisture stress. Moreover we have to take into account the soil air-

394 drying and rewetting pre-treatment that may have induced microbial community selection
395 [42] and a previous “Birch effect” [17]. By contrast, heat-drought disturbance combined with
396 shock of rewetting during restoration conditions induced a drastic initial biomass death and
397 increased shifts in taxonomic and catabolic structure and carbon allocation until the end of
398 our experiment with no resilience.

399 **4.2. Consequences of the different disturbance durations for microbial communities**

400 Increasing disturbance duration have induced an increased release of available organic matter
401 (as measured in DOC after the end of disturbance) and favoured C mineralization, as we
402 observed increase in basal respiration two days after the end of the disturbance.

403 Resilience of microbial parameters was inversely related to duration of disturbance. This was
404 particularly true for heat wave disturbances because, as we described, no resilience was
405 observed in D-H-21 disturbed soils. Indeed in these soils a drastic restructuring of microbial
406 communities and a collapse of microbial biomass were observed during the course of the
407 experiment, whereas microbial resilience was observed in the D-H-7 disturbed soils. This
408 study suggests that drought and heat wave induced gradients of reduction in microbial
409 biomass and selection pressure related to the duration of disturbance (Fig. 6C). Banning and
410 Murphy [43] similarly observed that resilience of soil SIR to heat disturbance was positively
411 related to the size of the initial microbial biomass. Our results further suggest that there was a
412 critical threshold of heat wave duration (somewhere between 7 and 21 days) above which no
413 resilience of microbial parameters was observed.

414 In some cases the 2-day disturbed soils presented stronger reactions in their microbial
415 communities than the 7-day disturbed soils. This may be because less than two days of
416 desiccation directly followed by watering was not sufficient to increase the proportion of
417 inactivated or tolerant microorganisms (dormant state or spores) by starvation [35] and/or to
418 induce an adaptation of microorganisms at the colony scale (e.g. biosynthesis of protective

419 EPS by microcolonies, [44]). Such inactive, tolerant and adapted microorganisms may be less
420 susceptible to the effects of rewetting disturbance [12].

421 Disturbance treatments and their duration were better differentiated during the course of the
422 experiment and at the end (resilience) than just after the end of the disturbance. Kuan et al.
423 [19] and Philippot et al. [45] observed, in studies respectively addressing the carbon and
424 nitrogen cycles, that resilience of microbial parameters could be an indicator of pre-exposure
425 to disturbance, but resistance was less. In some cases (especially the 21-day heat wave
426 treatments) we observed no resilience 63 days after restoration of environmental conditions.
427 Moreover, the disturbance seemed to have a lasting effect on some microbial parameters that
428 were dramatically altered by the end of experiment. Soil microbial communities may have
429 then undergone a drastic biomass killing inducing a shift in composition and a shift in
430 physiological traits (e.g. allocation of carbon demand for energy requirements versus growth)
431 as revealed by total EL-FAMES, SIR and qCO₂ measurements (Fig. 6). As a result, no
432 resilience seems to be possible [15].

433 In conclusion, these results represent a contribution to the understanding of soil microbial
434 community dynamics in the context of Mediterranean heat-wave disturbance in terms of loss
435 of microbial biomass, shifts in community structure and carbon allocation [4]. However, our
436 study is limited to one soil (although representative of Mediterranean agricultural soils) and
437 our experimental conditions do not allow us to draw conclusions about the long-term
438 dynamics of changes in soil microbial communities beyond 63 days after the end of the
439 longest disturbance. We must take into account that our microcosm experiments probably
440 induced a confinement effect and no possibility of microbial re-colonization from unperturbed
441 soil [46], interaction with plant growth and recovery of soil carbon supply [47], which could
442 contribute to some recovery of microbial biomass and functions of our high-disturbed soils. A

443 next step for studying heat wave impacts on soil microbial communities will be to introduce
444 experimentally the possibility of re-colonization from the unperturbed soil. Moreover to
445 conclude forward about the specific effect of high temperature into a drought combined to
446 heat disturbance, further experiments should assess the effects of high temperature with and
447 without drought on soil microbial communities.

448 We also need further detailed analyses in terms of community diversity (e.g. complementary
449 FAMES biomarkers as mycorrhizal indicators, biomolecular analysis as qPCR targeting
450 microbial groups) and functionality (e.g. bacterial and fungal contributions in carbon
451 allocation, [39]; soil enzyme activities targeting the C and N cycles, [11]). Incorporating this
452 knowledge into models would enhance our ability to predict soil ecosystem responses to
453 global climate change [15,13].

454 **5. Acknowledgements**

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1 Figure 1: A schematic diagram (not to scale), illustrating the time course of drying
2 stressdisturbance and drying-heating stressdisturbance (simulating drought and heat wave events
3 respectively) and the soil water potentials during the experiment.: At day 0 of the experiment, the
4 stressdisturbance is applied. Diamonds represent sampling dates, triangles represent dates chosen
5 to represent short-term impacts resistance and scoares dates chosen to represent resilience.
6

7 Figure 2: Percentage change relative to control soils for qCO₂ (Fig. 2A, Fig. 2B) and for DOC
8 (Fig. 2C, Fig. 2D), during the course of experiment (crosses indicate values from repeated
9 experiment;, day 23 DOC analyseis were lost). Vertical bars indicate the standard deviation (n=4
10 for qCO₂ and n=3 for DOC values).

11 Stars (*) indicate significant difference between the control and treated microcosms (Mann-
12 Whitney tests performed on the parameter raw data, $p \leq 0.05$).

Mis en forme : Couleur de police :
Automatique

14 Figure 3: Factorial maps and factors effects (F values and probability, * for $p < 0.05$ and ** for
15 $p < 0.01$) from redundancy analyses performed on CLPPs., Fig. 3A and considering as source of
16 variation as stressdisturbance treatment (C=control; D-25= Drying stressdisturbance; D-50H=
17 Drying- heating stressdisturbance) and disturbance duration (2; 7; and 21 days). Fig. 3B
18 considering source of variation as disturbance treatment and recovery time considered after the
19 disturbance (days 0 to 84 after the start of the disturbances). Each triangle and circle is the
20 barycentre coordinate from replicates of each perturbation treatment, perturbation duration or
21 recovery time.(Fig. 3A and Fig. 3B, representss the correlation plot between the RDA axes and
22 the carbon sources selected for SIR) or stress treatment and the recovery time considered after the
23 stress (Fig. 3C and Fig. 3D, represents the correlation plot between the RDA axes and the carbon
24 sources selected for SIR). [Pas très clair. Diviser en deux ou trios phrases?]

Mis en forme : Non Surlignage

Mis en forme : Non Surlignage

Mis en forme : Anglais (États Unis)

Comment citer ce document :

Berard, A., Bouchet, T., Sevenier, G., Pablo, A.-L., Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology, 7 (6), 333-342. DOI : 10.1016/j.ejsobi.2011.08.004

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25

26 Figure 4: Percentage change relative to control soils for respiration induced by substrate chemical
27 groups induced respiration. Treatments: 21-day dDrying 21 days duration stress treatment (D-
28 25-21, Fig. 4A), drying-heating 21- day drying-heating s duration stress treatment (D-50-21, Fig.
29 4B). Open White symbols indicate values of the repeated experiment (Fig. 4B). Vertical bars
30 indicate the standard deviation (n=4).

31

32 Figure 54: Factorial maps and factor effects (F values and probability, * for $p < 0.05$ and ** for
33 $p < 0.01$) from redundancy analyses performed on EL-FAMEs. Fig. 4A considering source of
34 variation as disturbance treatment (C=control; D= Drying disturbance; D-H= Drying-heating
35 disturbance) and disturbance duration (7; and 21 days). Fig. 5B considering source of variation as
36 disturbance treatment and recovery time considered after the disturbance (days 0 to 84 after the
37 start of the disturbances). Each triangle and circle is the barycentre coordinate from replicates of
38 each perturbation treatment, perturbation duration or recovery time.

39 Factorial maps and factors effects (F values and probability, * for $p < 0.05$ and ** for $p < 0.01$)
40 from redundancy analyses performed on FAMEs, and considering as source of variation as stress
41 treatment and duration (Fig. 5A and Fig. 5B, represents the correlation plot between the RDA
42 axes and the FAMEs identified for more than 5%) or stress treatment and the evolution time
43 considered after the stress (Fig. 5C and Fig. 5D, represents the correlation plot between the RDA
44 axes and the FAMEs identified for more than 5%). C=control; D-25= Drying stress; D-50=
45 Drying heating stress (7 and 21 days duration treatments). [En faire plusieurs phrases?]

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Berard, A., Bouchet, T., Sevenier, G., Pablo, A.-L., Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology, 7 (6), 333-342. DOI : 10.1016/j.ejsobi.2011.08.004

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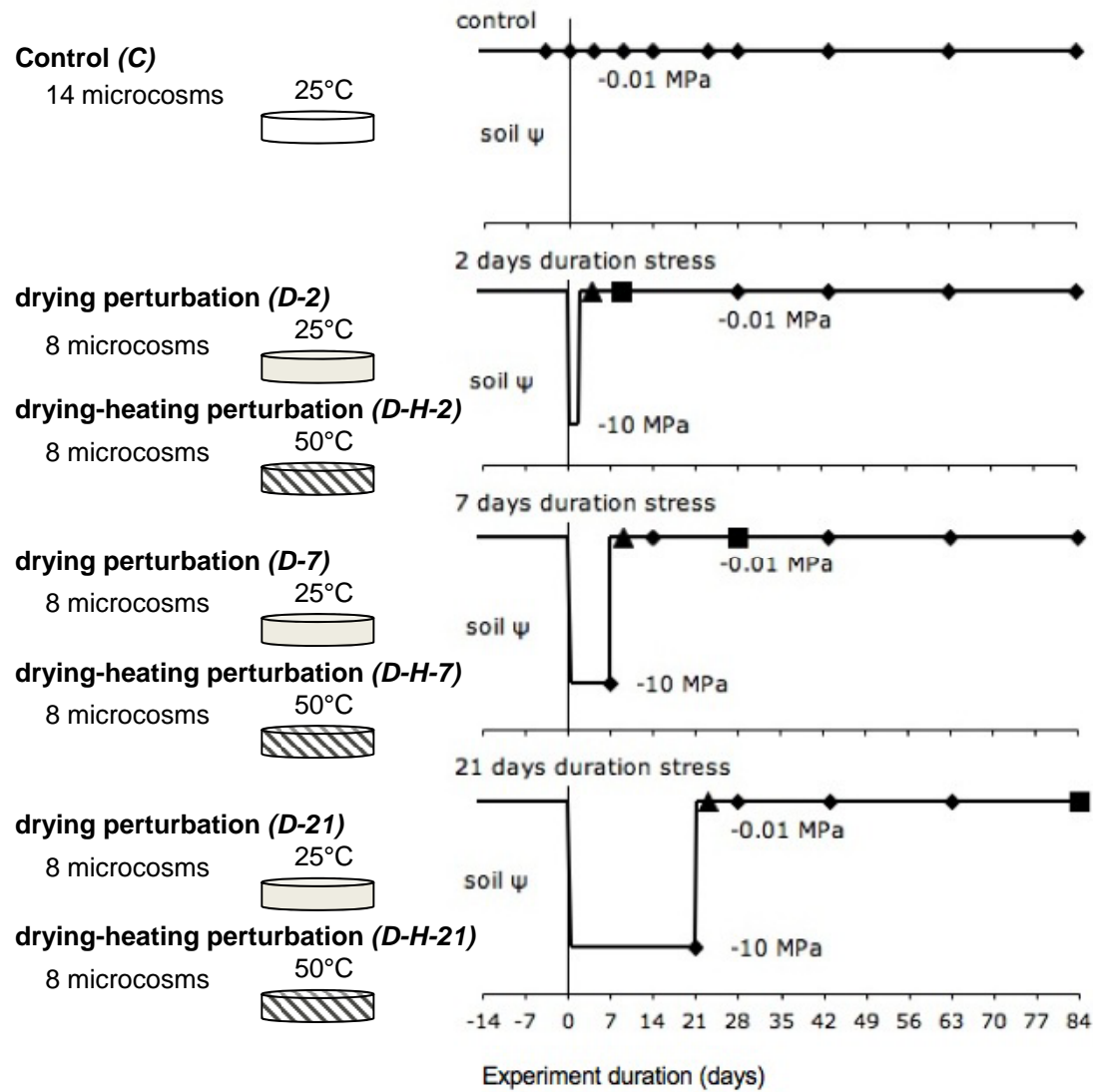
48 | Figure 65: Percentage change relative to control soils microbial EL-FAME biomarkers.
49 | Treatments: Drying 21-day drying days duration stressdisturbance treatment (D-25-21, Fig.
50 | 6A5A), drying-heating 21-day drying-heating days duration stressdisturbance treatment (D-50H-
51 | 21, Fig. 56B). Vertical bars indicate the the standard deviation (n=3).
52 |

53 | Figure 76: Schematic representation of the soil microbial community response to 21-day the heat
54 | -wave stressdisturbance (Fig. 7A6A) and drought stressdisturbance (Fig. 7B6B) 21 days stresses.
55 | Illustration of the impacts (measured at “resilience dates”) of stressdisturbance duration on soil
56 | samples subjected mitted to heat wave (Fig. 7C6C). Resilience dates: day 8 for the 2 day-
57 | disturbance; day 28 for the 7 day-disturbance; day 84 for the 21 day-disturbance.

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Berard, A., Bouchet, T., Sevenier, G., Pablo, A.-L., Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology, 7 (6), 333-342. DOI : 10.1016/j.ejsobi.2011.08.004



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Table 1: Mean values of biotic parameters and DOC of control soil, measured during the experiment (standard deviation in brackets; n=3 for ELFAMEs and DOC, n=4 for respiration, SIR measurements and qCO₂). FAMES biomarkers of specific functional groups: Actinobacteria (ACT); Fungi (FUNG); Gram-positive bacteria (G+), Gram negative bacteria (G-).

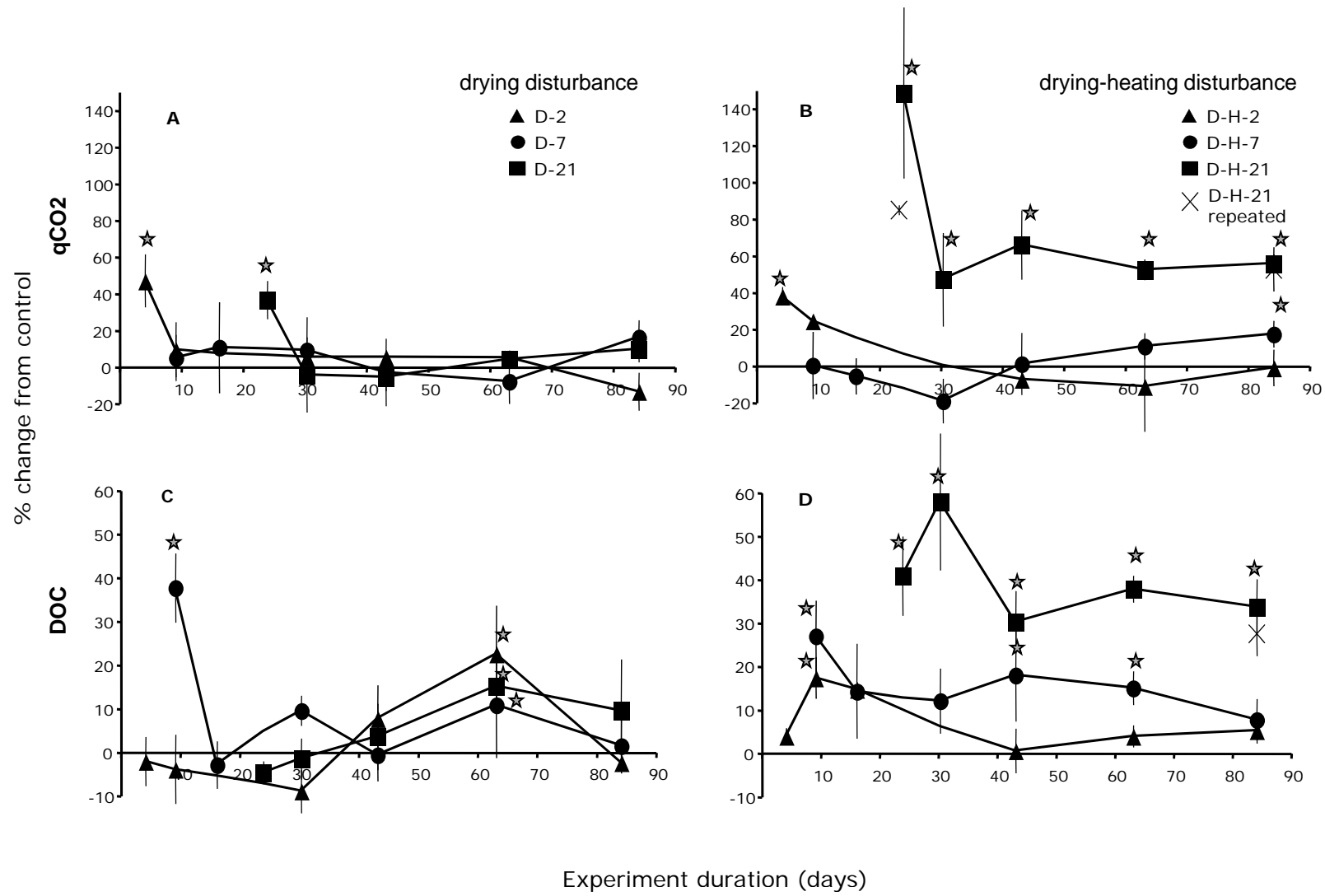
Days of experiment	0	4	9	21	23	28	84
Basal respiration (µgC-CO ₂ g ⁻¹ dry soil h ⁻¹)	0.84 (0.19)	0.72 (0.02)	0.77 (0.07)		0.25 (0.03)	0.26 (0.04)	0.60 (0.00)
SIR Glucose (GIR) (µgC-CO ₂ g ⁻¹ dry soil h ⁻¹)	1.07 (0.13)	1.39 (0.17)	1.21 (0.42)		0.76 (0.12)	0.68 (0.20)	1.41 (0.08)
qCO ₂	0.79 (0.14)	0.53 (0.08)	0.68 (0.19)		0.33 (0.07)	0.39 (0.06)	0.43 (0.02)
DOC (µgC g ⁻¹ dry soil)	85.75 (0.75)	83.05 (9.47)	68.75 (2.26)		96.98 (0.31)	86.56 (6.96)	95.14 (6.55)
Total FAMES (nmol g ⁻¹ dry soil)	63.44 (20.84)	62.85 (2.97)	68.89 (5.46)	50.29 (25.08)	66.65 (6.43)	69.62 (15.57)	53.36 (4.71)
ACT (nmol g ⁻¹ dry soil)	5.38 (1.59)	4.50 (0.51)	5.15 (0.84)	4.31 (1.00)	4.48 (0.30)	4.63 (0.58)	4.14 (0.35)
FUNG (nmol g ⁻¹ dry soil)	7.11 (1.49)	8.15 (1.37)	7.82 (1.35)	8.44 (3.04)	9.48 (2.38)	7.67 (0.36)	6.44 (0.35)
G + (nmol g ⁻¹ dry soil)	15.69 (3.50)	16.30 (2.25)	15.53 (2.89)	12.34 (5.45)	15.46 (1.98)	16.24 (0.40)	15.42 (0.81)
G - (nmol g ⁻¹ dry soil)	35.26 (16.35)	33.90 (2.15)	40.39 (9.24)	27.14 (15.54)	37.24 (7.10)	41.07 (15.54)	27.36 (4.10)
Saturated to unsaturated fatty acids	0.62 (0.09)	0.64 (0.06)	0.59 (0.04)	0.52 (0.16)	0.58 (0.12)	0.63 (0.00)	0.67 (0.06)

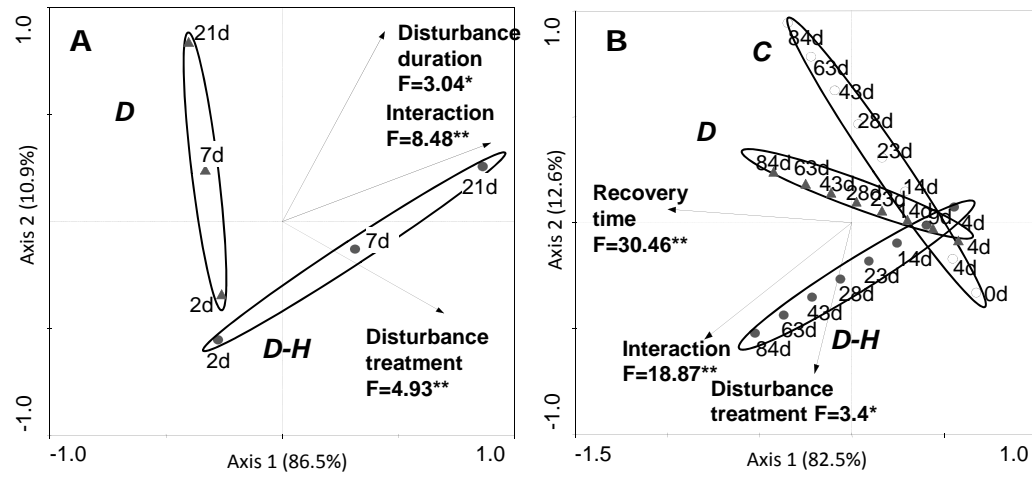
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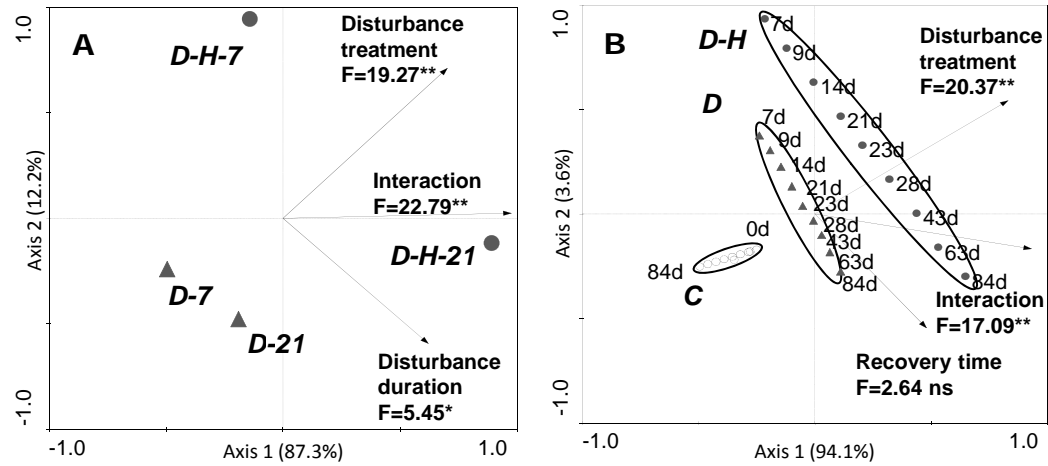
Berard, A., Bouchet, T., Sevenier, G., Pablo, A.-L., Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology, 7 (6), 333-342. DOI : 10.1016/j.ejsobi.2011.08.004

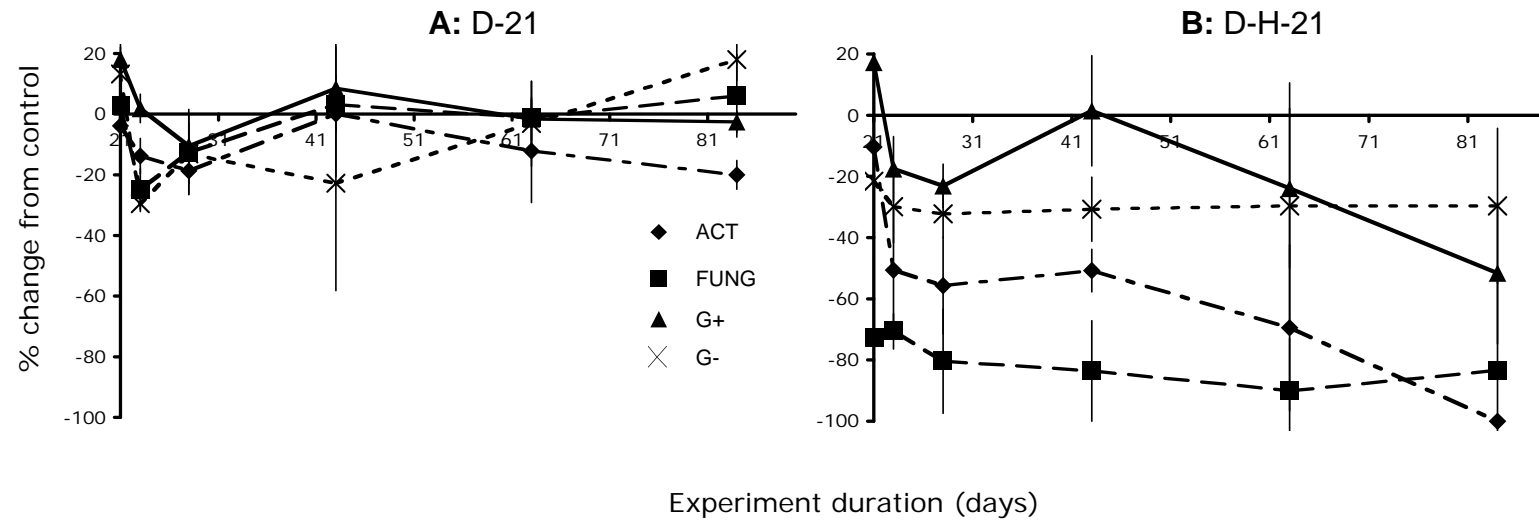
Table 2: Mean percentage change relative to control (standard deviation in brackets) for rate of soil respiration (n=4), SIR Glucose (n=4), concentration of total FAMEs (n=3), ratio saturated to unsaturated fatty acids (n=3) and Bray and Curtis Indices (BCI) for CLPP (n=4) and FAMEs (n=3), measured 2 days after the end of perturbation and 3 times perturbation duration after end of perturbation for Resistance and Resilience respectively.

Parameters	Measurements	D-2	D-H-2	D-7	D-H-7	D-21	D-H-21	D-H-21 repeated
Days of experiment	Resistance	4	4	9	9	23	23	23
	Resilience	9	9	28	28	84	84	84
Basal respiration	Resistance	-3.0 (12.9)	-3.8 (6.5)	0.9 (16.0)	14.1 (16.3)	124.0 (13.1)	140.8 (31.9)	51.6 (12.0)
	Resilience	17.7 (12.4)	16.7 (12.6)	36.7 (10.6)	-10.8 (16.3)	6.5 (4.1)	-14.4 (0.9)	-13.0 (3.3)
SIR Glucose	Resistance	-35.3 (3.6)	-31.4 (5.8)	-15.1 (6.4)	-11.8 (6.0)	-23.8 (2.9)	-31.3 (7.6)	-18.9 (6.5)
	Resilience	-8.9 (6.8)	-13.2 (9.7)	16.1 (5.7)	3.0 (5.0)	-3.5 (5.1)	-45.3 (2.2)	-34.03 (6.3)
CLPP-BCI	Resistance	0.903	0.908	0.863	0.917	0.850	0.883	0.937
	Resilience	0.918	0.873	0.910	0.864	0.955	0.854	0.843
Total FAMEs	Resistance			-18.8 (9.5)	-8.8 (24.2)	-20.5 (3.7)	-34.2 (5.3)	
	Resilience			-0.2 (19.1)	-19.4 (27.4)	7.7 (6.3)	-47.9 (23.0)	
Saturated to unsaturated fatty acids	Resistance			-1.1 (17.5)	24.7 (15.4)	9.1 (1.9)	33.4 (6.9)	
	Resilience			2.5 (11.4)	6.9 (6.5)	1.3 (3.3)	-24.0 (35.7)	
FAMEs-BCI	Resistance			0.855	0.836	0.828	0.736	
	Resilience			0.873	0.799	0.851	0.545	

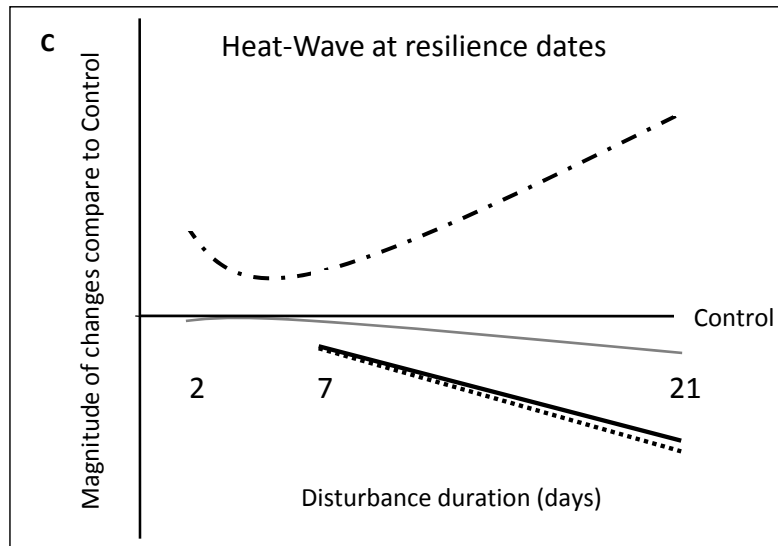
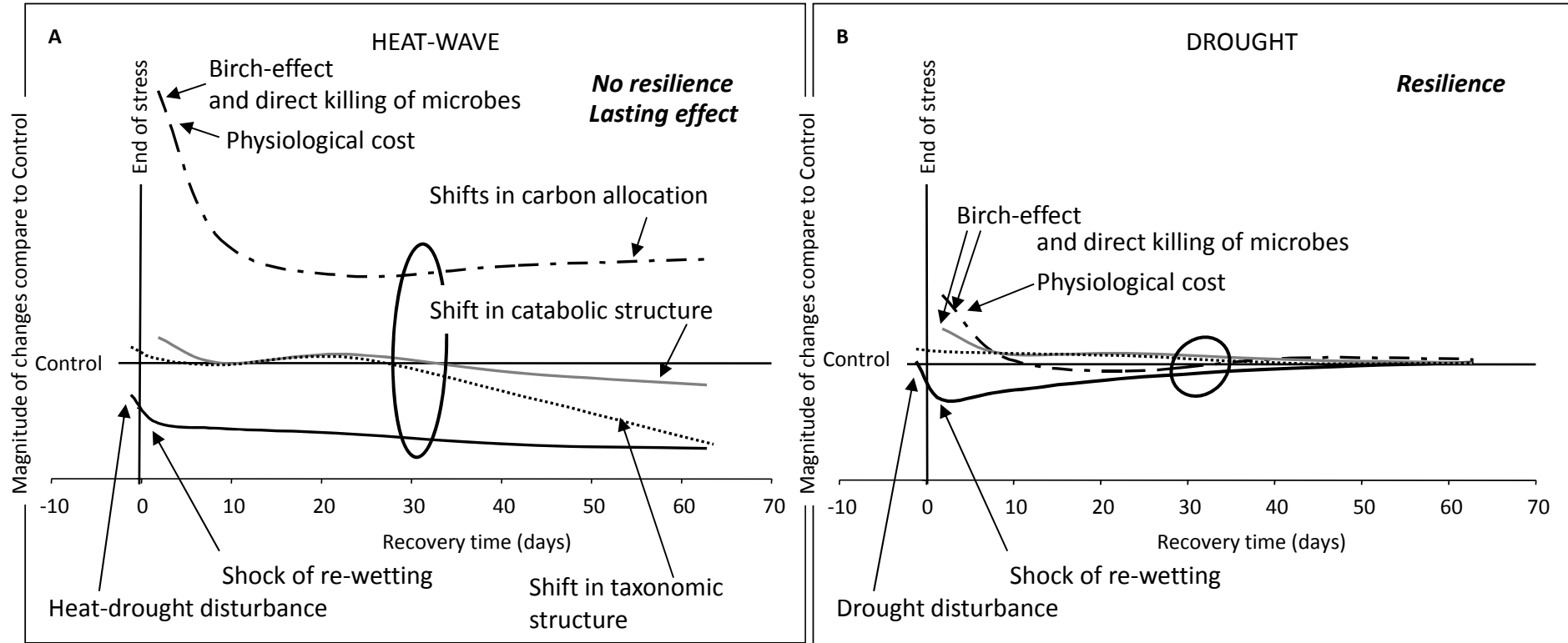








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- Total biomass (FAMEs)
- - - Catabolic quotient qCO2
- Bray and Curtis indice for CLPP
- Bray and Curtis indice for FAMEs