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Abstract

Mediterranean forests will experience more frequent and intense drought periods and extreme rainfall events in the coming decades. Concomitantly, drought-induced forest die-off is likely to increase. Changes in rainfall patterns and forest die-off directly influence soil microbial communities and activity and, consequently, carbon (C) and nitrogen (N) turnover, but their interactive effects have not yet been explored. We investigated the short-, and the long-term interactive effects of forest die-off and changes in water regimes on soil C and N mineralization rates of a Mediterranean woodland. Soil samples collected under and out of the influence of holm oak (*Quercus ilex*) trees with different defoliation degrees (six healthy, six affected and six dead) were incubated under two contrasting water regimes (i.e. drying-rewetting cycles vs. constant soil moisture). Potential soil C and N mineralization responded differently to changes in soil water regime, with an overall 55% increase in C mineralization and a 22% decrease in N mineralization in the drying-rewetting cycle treatment compared to the constant moisture treatment. Holm oak decline decreased the response of C mineralization while increased the response of N mineralization to the drying-rewetting cycles at both the short- and the long-term. Moreover, N turnover showed a higher sensitivity to these environmental disturbances than that of C during most of the year. Our study provides solid evidence that increasing drying-rewetting cycles can result in a decoupling of soil C and N cycles in Mediterranean forests and that forest die-off might enhance this decoupling at both the short- and the long-term, with important implications for the ecosystem functioning.

Keywords	Mediterranean forest; tree defoliation and mortality; microbial functioning; C cycling; N cycling; water regime
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Dear Editor,

Please find attached the manuscript entitled "Interactive effects of forest die-off and changing water regimes on C and N mineralization".

Mediterranean forests are considered biodiversity hotspots and are representative of one of the most widely distributed semi-arid areas throughout the world. These forests are, and will keep suffering, changes in the precipitation regime with more frequent and intense drought periods and extreme rainfall events and, therefore, increased tree defoliation and mortality (forest die-off). Both, changes in precipitation patterns and forest die-off will have direct consequences on soil microbial communities and activity and, consequently, on C and N turnover, but the interactive effect of both is still unclear. Herein we investigated the short- and long-term interactive effects of forest die-off and changes in soil water regime on soil C and N mineralization rates of a Mediterranean woodland.

Briefly, our results demonstrate that increasing drying-rewetting cycles might decouple soil C and N cycles in Mediterranean forests and that forest die-off might enhance this decoupling at both short- and long-term scales, with important implications for the ecosystem C and N budgets.

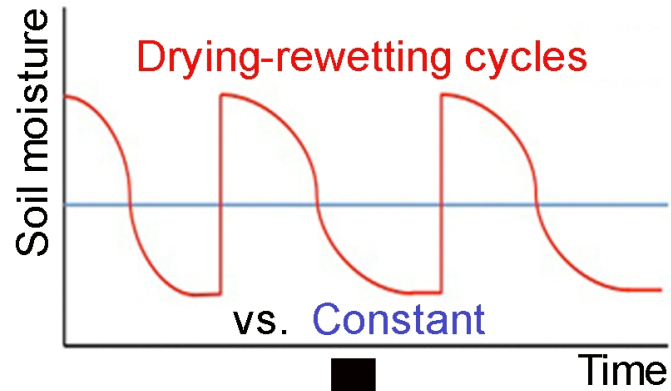
We believe that the submitted manuscript falls within the scope of *Geoderma*, and that the contents of the manuscript will be of interest for most of its readers. The enclosed work has not been published or accepted for publication, and is not under consideration for publication in another journal or book. All the co-authors have read and approved the submitted version of the manuscript.

Best regards,

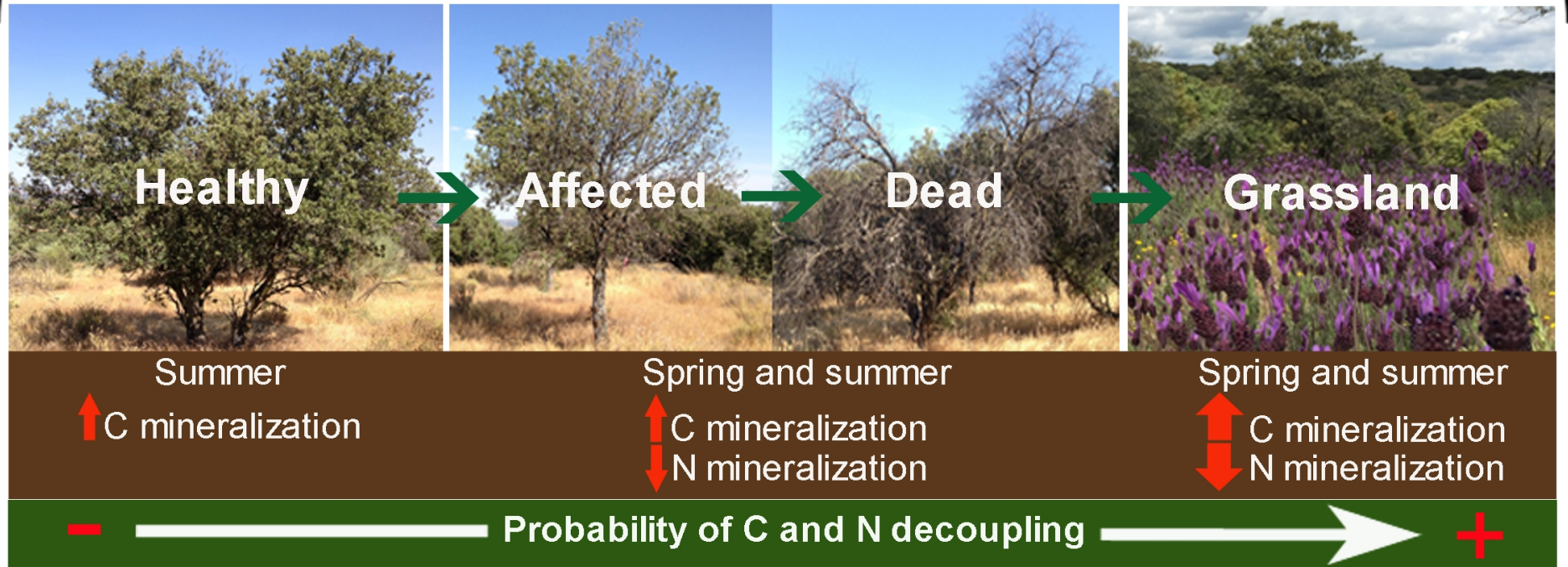
Alexandra Rodríguez (arp@uc.pt), on behalf of all co-authors.

Highlights

- Drying-rewetting cycles decouples C and N cycling in Mediterranean forests
- Drying-rewetting cycles increase soil C mineralization
- Drying-rewetting cycles decrease soil N mineralization
- Forest die-off enhances the probability of C and N decoupling
- N cycle is more sensitive than C cycle to water regimes changes and forest die-off



Forest die-off



1 **Title:** Interactive effects of forest die-off and changing water regimes on C and N
2 mineralization.

3

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28 **Type of article:** Regular paper.

29 **Abstract**

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31 extreme rainfall events in the coming decades. Concomitantly, drought-induced forest die-
32 off is likely to increase. Changes in rainfall patterns and forest die-off directly influence soil
33 microbial communities and activity and, consequently, carbon (C) and nitrogen (N) turnover,
34 but their interactive effects have not yet been explored. We investigated the short-, and the
35 long-term interactive effects of forest die-off and changes in water regimes on soil C and N
36 mineralization rates of a Mediterranean woodland. Soil samples collected under and out of
37 the influence of holm oak (*Quercus ilex*) trees with different defoliation degrees (six healthy,
38 six affected and six dead) were incubated under two contrasting water regimes (i.e. drying-
39 rewetting cycles vs. constant soil moisture). Potential soil C and N mineralization responded
40 differently to changes in soil water regime, with an overall 55% increase in C mineralization
41 and a 22% decrease in N mineralization in the drying-rewetting cycle treatment compared to
42 the constant moisture treatment. Holm oak decline decreased the response of C
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45 to these environmental disturbances than that of C during most of the year. Our study
46 provides solid evidence that increasing drying-rewetting cycles can result in a decoupling of
47 soil C and N cycles in Mediterranean forests and that forest die-off might enhance this
48 decoupling at both the short- and the long-term, with important implications for the
49 ecosystem functioning.

50

51 **Keywords:** Mediterranean forest; tree defoliation and mortality; microbial functioning; C
52 cycling; N cycling; water regime

53 **1. Introduction**

54 Soils represent the largest carbon (C) and nitrogen (N) pools in forest ecosystems
55 (Schlesinger and Bernhardt, 2013). When a dry soil is rewetted, a pulse of microbial activity
56 occurs, with important consequences for soil C and N cycling at ecosystem level (Blazewicz
57 et al., 2014; Borken and Matzner, 2009; Jarvis et al., 2007). This microbial activity pulse,
58 named the *Birch effect* after one of its first observers (Birch, 1958), can be a major
59 contribution to ecosystem C release into the atmosphere (Jenerette et al., 2008). The size of
60 the pulse depends on the intensity and duration of the rainfall and the previous drought event,
61 as well as on the vegetation and soil organic matter content and quality (Huxman et al., 2004;
62 Meisner et al., 2015; Morillas et al., 2017; Song et al., 2017; Wang et al., 2016). Global
63 warming circulation models predict changes in precipitation regimes including more frequent
64 and intense droughts as well as increased extreme rainfall events (IPCC, 2013). These
65 changes will be particularly critical in the Mediterranean region, characterized by a large
66 intra-annual variation in soil water content, which largely regulates ecosystem functioning
67 (Gallardo et al., 2009). Whilst recent studies have suggested that small rainfall pulses and
68 drying-rewetting processes may be the main driver of soil C and N cycling in Mediterranean
69 environments (Rey et al., 2017; Song et al., 2017; Wang et al., 2016), the effects of multiple
70 drying-rewetting cycles on both C and N mineralization rates of Mediterranean forest soils
71 have not yet been well elucidated (Wang et al., 2016).

72 Mediterranean forests are representative of one of the most widely distributed semi-
73 arid areas throughout the world (Jarvis et al., 2007) and are considered biodiversity hotspots.
74 Intensified droughts are increasing tree defoliation and mortality in these forests (Carnicer et
75 al., 2011; Lloret et al., 2004). On the short-term, soil microbial-driven C and N
76 mineralization could rise followed by rapid N losses due to stimulated decomposition of

77 litter, roots and dead wood (Edburg et al., 2012; Lloret et al., 2014; Xiong et al., 2011). On
78 the long-term, repeated drought-induced mortality events, along with an unsuccessful
79 recruitment of the dominant tree species in these forests may lead to a vegetation succession
80 process where trees would be replaced by understory species (Ibáñez et al., 2015; Saura-Mas
81 et al., 2014). This succession process would have an impact on ecosystem functioning, and
82 therefore on critical local and global ecosystem services, even more severe than the short-
83 term direct effects of tree defoliation and mortality (Anadón et al., 2014; Ávila et al., 2016).
84 However, the impacts of forest die-off on soil microbial functioning both at the short- and
85 long-term are still far from being understood (Ávila et al., 2016; Rodríguez et al., 2017).
86 More importantly, although there is increasing evidence that multiple environmental
87 disturbances can generate effects that are not predictable from single-factor studies (Doblas-
88 Miranda et al., 2017; Matesanz et al., 2009; Morillas et al., 2015), how forest die-off may
89 influence the response of microbial functioning to changing rainfall patterns has not been yet
90 studied.

91 Herein, we aimed to investigate the short- and long-term interactive effects of forest
92 die-off and changes in water regime on soil microbial activity, as determined by C and N
93 mineralization rates, of a holm oak (*Quercus ilex*) woodland. To do so, we collected and
94 biogeochemically characterized soil samples under (holm oak ecotype) and out (grassland
95 ecotype) of the influence of selected holm oak trees with different defoliation degrees. Then,
96 we measured soil potential C and N mineralization rates under two different water regimes
97 (i.e. constant soil moisture vs. drying-rewetting cycles). The comparison among samples
98 collected in the holm oak ecotype allowed us to explore the short-term effects of holm oak
99 decline (HOD). The comparison among samples collected in both (holm oak vs. grassland)
100 ecotypes allowed us to explore the HOD long-term effects under a plant succession scenery

101 (Rodríguez et al. 2017). Based on previous studies that showed different sensitivity of N and
102 C-related processes to disturbances (e.g. Durán et al., 2013; Morillas et al., 2015, 2017;
103 Rodríguez et al., 2017; Tan and Wang, 2016), we hypothesized that soil C and N
104 mineralization would response differently to changes in water regimes, as well as to the
105 interaction of HOD and soil water regime. Further, given the seasonality in soil water
106 availability of Mediterranean ecosystems, and its influence on biogeochemical cycles, we
107 hypothesized that the response of C and N mineralization to these environmental disturbances
108 would vary depending on the time of the year (spring vs. summer).

109

110 **2. Material and Methods**

111 *2.1 Study site*

112 The study was carried out in a holm oak woodland located in the central part of the
113 Iberian Peninsula, southwest of Madrid (40°23'N, 4°11'W; 630-660 *m* above sea level).
114 Climate is continental Mediterranean with mean annual temperature of 15 °C and mean
115 annual precipitation of 558 mm (Ninyerola et al., 2005). Most rainfall concentrates from
116 autumn to spring, while summers are warm and dry. Soil is a Cambisol, sandy and slightly
117 acid (pH ~ 6.3), with low total C and N content (Table 1). Aboveground vegetation is
118 characterized by a tree density of ~180 trees ha⁻¹, mostly composed of *Quercus ilex* ssp.
119 *Ballota* L. (holm oak) with scarce *Juniperus oxycedrus* Sibth. & Sm (cedar). The understory
120 is dominated by *Retama sphaerocarpa* L., *Lavandula stoechas* ssp. *pedunculata* (Mill.)
121 Samp. ex Rozeira, and diverse pasture species (see Rodríguez et al. 2017 for more
122 information about the study site).

123 This region suffered a severe drought in 2005 (European Environment Agency,
124 2008), with a 55% decrease in the average annual rainfall. This drought resulted in a strong
125 event of holm oak defoliation (around 20-30% of the total population) and mortality (15%)
126 that persists (Valladares, unpublished data). Consequently, this woodland shows a
127 successional chronosequence that goes from a range of holm oak trees with different
128 defoliation degree (holm oak ecotype) to a grassland ecotype, with contrasting soil
129 biogeochemistry and microbial diversity characteristics (Table 1).

130

131 *2.2 Experimental design*

132 We selected 18 holm oak trees of similar size based on its crown defoliation degree
133 (six healthy, six affected, and six dead trees) separated at least 10 m from other trees. For
134 each tree, we established a 5-m, north-facing transect with two sampling points, one under
135 the tree canopy, at 0.3 m from the trunk (rhizosphere of holm oak ecotype), and one 5 m
136 away from the trunk (rhizosphere of grassland ecotype), out of the influence of the tree
137 (Rodríguez et al., 2011; Tang and Baldocchi, 2005). This paired-point design has been
138 successfully used in the past (Rodríguez et al., 2017), and allowed us to explore both the
139 short- and the long-term effects of holm oak decline (HOD). Moreover, it allowed us to
140 measure the effect of HOD while distinguishing it from other confounding factors such as
141 the inherent spatial variability of soils (Barba et al., 2013). At the end of spring (June) and
142 summer (September) of 2013, one soil sample was collected from the first 10 cm of the soil
143 profile in each sampling point using a metal corer of 5 cm (i.d.), and kept at 4 °C until
144 analysis. For more information about the experimental design see Rodríguez et al. (2017).

145

146 *2.3 Soil preparation*

147 Soil samples were sieved (2 mm mesh size), homogenized under field moist
148 conditions and analyzed for soil water content (SWC) by oven-drying a subsample of 5 g at
149 60 °C to constant weight. Water holding capacity (WHC) was determined following Rey and
150 Jarvis (2006).

151

152 *2.4 Soil biogeochemistry and microbial diversity*

153 Soil total C and N content was determined by dry combustion with an elemental
154 analyzer (LECO TruSpec CN). Given the temporal stability of these two variables and the
155 proximity of the spring and summer samplings, these analyses were carried out only for the
156 spring samples. Soil inorganic N, microbial biomass and functional alpha-diversity of soil
157 bacterial communities were estimated in both spring and summer soil samples as described
158 in Rodríguez et al. (2017). Briefly, soil inorganic N was extracted by shaking fresh soil
159 subsamples with 0.5 M K₂SO₄ in an orbital shaker and filtering the suspension through a 0.45
160 mm Millipore filter. Then, we used these extracts to colorimetrically determine the amount
161 of NH₄⁺-N and NO₃⁻-N (Durán et al., 2009). Soil microbial biomass was estimated by using
162 the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978). We estimated
163 the functional alpha-diversity and richness of soil bacterial communities by using Biolog®
164 EcoPlates™ (BIOLOG Inc., Hayward, CA) to assess the community-level physiological
165 profiles (CLPP), following Flores-Rentería et al. (2016). Biolog EcoPlates is a method based
166 on carbon substrate utilization recognized as a useful tool for comparing microbial
167 communities . Briefly, soil microorganisms were extracted by shaking fresh soil subsamples
168 with sterile 0.8% saline solution (NaCl) in an orbital shaker for 20 min, and left to stand at
169 room temperature for 30 min. The supernatant was diluted into the sterile saline solution to
170 a 1:1000 final dilution, mixed for 30 s and left to stand for 10 min. Then, a 100 ml aliquot of

171 each diluted solution was added to each of 96 wells (arranged by triplicate for each substrate).
172 Plates were incubated at 28 °C in a humidity-saturated environment and darkness. Optical
173 density (590 nm), which is indicative of carbon-source utilization, was measured and
174 recorded every 24 h during 7 days using a Victor3 microplate reader (Perkin-Elmer Life
175 Sciences, Massachusetts, USA). Optical density (absorbance) at the day the plate reached the
176 asymptote (120 h) was the value used in all posterior analyses. The three values for each
177 individual substrate within a plate were averaged. Functional alpha-diversity, i.e. how
178 diversified the species are within a site, of bacterial communities was evaluated through both
179 the Shannon index (H'_{bact}) and richness (S_{bact}). Shannon index was calculated as follows
180 (Formula 1):

181

$$182 \quad \text{Shannon } (H') = - \sum_{i=1}^S \frac{n_i}{N} \times \ln\left(\frac{n_i}{N}\right) \quad (1)$$

183

184 where n_i is the absorbance of a specific well (C substrate) and N is the whole absorbance of
185 the plate. Functional richness is calculated as the total number of C substrate catalyzed.

186

187 *2.5 Soil incubation experiment: Potential C and N mineralization rates*

188 We carried out 40-day, in darkness laboratory incubations (25 °C) of the 18 soil
189 samples collected under the holm oak (H) and under the grassland (G) ecotype both in spring
190 and summer. Subsamples (50 g) of each soil sample were incubated at one of the two different
191 water regimes: a constant regime with steady soil moisture at 25% WHC of soil samples (i.e.
192 10% and 9% SWC for H and G samples, respectively), and a cycles regime with three 13-
193 days drying-rewetting cycles, in which soil water content oscillated between 40% WHC

194 (16% and 14% SWC for H and G samples, respectively) and 10% WHC (4% and 3% SWC
195 for H and G samples, respectively) (Figure 1). Rewetting events consisted in adding ~1.8 mm
196 of water to reach the 40% WHC. The drying-rewetting cycles regime resembles the natural
197 annual oscillation in soil water content at the study site, while the soil water content of the
198 constant regime represents the annual mean encountered at the site. All soil samples received
199 the same amount of water in both regimes.

200 To estimate potential C mineralization rates, we periodically measured the
201 heterotrophic respiration rate (R_H) during the incubation by placing each sample inside a 1L
202 gastight glass jar with a lid connected to a portable, closed-chamber soil respiration system
203 (EGM-4, PP systems, MA, USA) during 60 s. Then, we used the ideal gas law equation to
204 convert and extrapolate the net CO₂ increase (ppm) to mass of C (m) in the headspace of the
205 jar (Formula 2):

206

$$207 \quad m = \frac{ppm \times P \times V \times M}{R \times T} \quad (2)$$

208

209 where P and V are, respectively, the air pressure (ATM) and the known headspace
210 volume in the jar (L), M is the atomic mass of carbon (g mol⁻¹), R is the universal constant
211 of gases (0.08206 ATM L mol⁻¹ K⁻¹) and T is the temperature (K) at the measurement time.

212 In both water regimes, we carried out R_H measurements before, and three hours after
213 the first soil rewetting (Day 0), as well as on days 1, 2, 5, 7, 12, 19, 26, 33 and 40. In the case
214 of the drying-rewetting cycles regime, we rewetted soils on days 12 and 26 (after they reached
215 10% WHC) and measured R_H three hours after rewetting and on days 13, 14, 27 and 28. We
216 estimated the potential C mineralization rate for each water regime (C_m) by interpolation of

217 R_H between measurement dates. Potential C mineralization was normalized both by dry soil
218 mass ($\text{mg C-CO}_2 \text{ kg}^{-1} \text{ soil day}^{-1}$) and by soil initial C content ($\text{g C-CO}_2 \text{ kg}^{-1} \text{ C day}^{-1}$).

219

220 We estimated the pulse effect of each rewetting event on microbial respiration
221 (R_{Hpulse}) as follows (Formula 3):

222

$$223 \quad R_{Hpulse} = R_{Hmax} - R_{H0} \quad (3)$$

224

225 where R_{Hmax} is the maximum heterotrophic respiration rate we observed after the
226 rewetting event and R_{H0} is the heterotrophic respiration rate measured right before the
227 rewetting event.

228 Potential N mineralization rates (N_m) for each water regime were estimated by
229 assessing the increase in total inorganic N over the incubation period. To do so, we measured
230 soil inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) before and after the incubation of soil samples.
231 Inorganic N was extracted by shaking fresh soil subsamples (5 g) with 25 ml of 0.5M K_2SO_4
232 for 1 h at 200 rpm in an orbital shaker and filtering the suspension through a 0.45 mm
233 Millipore filter. Then, we used these extracts to colorimetrically determine the amount of
234 $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ as described by (Durán et al., 2009). Potential N mineralization was
235 normalized both by dry soil mass ($\text{mg N kg}^{-1} \text{ soil day}^{-1}$) and by initial C content ($\text{mg N kg}^{-1} \text{ C}$
236 day^{-1}).

237 Finally, to explore the interactive effect of holm oak decline (HOD) and the change
238 in soil water regime from constant moisture to drying-rewetting cycles, we estimated the
239 percent change in C_m and N_m for each sample as follows (Formula 4):

240

241
$$\text{Percent change} = \left(\frac{X_{cy} - X_{cte}}{X_{cte}} \right) * 100 \quad (4)$$

242

243 where X_{cy} is the mineralization rate in the cycles regime and X_{cte} is the mineralization
244 rate in the constant regime.

245

246 *2.6 Statistical analyses*

247 The effects of defoliation degree and ecotype, as well as the interaction among them,
248 on soil biogeochemistry and bacterial functional alpha-diversity variables were assessed for
249 each season using linear mixed-effects models. Tree identity was considered as a random
250 factor to account for the likely spatial dependency of the two ecotypes (holm oak and
251 grassland) selected for the same tree. Similarly, the effects of water regime, defoliation
252 degree and ecotype, as well as the interactions among them, on potential C and N
253 mineralization rates (C_m and N_m , respectively) were assessed for each season using liner
254 mixed-effect models with tree as a random factor. We also used linear mixed-effects models
255 to test the general effect of defoliation degree on C_m through the incubation for each season
256 and ecotype separately, and the general effect of defoliation degree, ecotype and season on
257 the percent change of C_m and N_m and R_{Hpulse} . Temporal dependencies (repeated measures)
258 were considered by using tree as a random factor.

259

260 Simultaneous tests for general linear hypotheses (multiple comparisons of means:
261 Tukey contrasts) were performed to test pairwise statistical differences between ecotypes for
262 each defoliation degree, season and water regime. Subsequently, and due to the strong

263 influence of the ecotype factor, we used one-way ANOVA to evaluate the effect of
264 defoliation degree within each ecotype, water regime and season level separately, as well as
265 on the potential C mineralization rate for each day individually. Pairwise statistical
266 differences among defoliation degrees were tested using the Tukey's test.

267 All statistical analyses were performed in R 3.1.1 (R Core Team, 2014). Linear
268 mixed-effects models and simultaneous tests for general linear hypotheses were performed
269 using the statistical package lme4 (Bates et al., 2015) and multcomp (Bretz et al., 2011),
270 respectively.

271

272 **3. Results**

273 *3.1 Effect of defoliation and ecotype on soil biogeochemistry and microbial functional* 274 *diversity*

275 Defoliation significantly decreased $\text{NH}_4^+\text{-N}$ and bacterial functional richness (S_{bact})
276 in soil samples collected under the holm oak ecotype in summer (Table 1). Neither total C
277 and N nor microbial biomass (SIR) were significantly affected by the defoliation degree
278 (Table 1). All variables were significantly higher in the holm oak than in the grassland
279 ecotype (Table 1), except for the $\text{NO}_3^-\text{-N}$ concentration of soil samples collected in summer,
280 which did not show significant differences.

281

282 *3.2 Effect of water regime on potential C and N mineralization rates*

283 Water regime had significant and contrasting effects on C_m and N_m (Table 2). Drying-
284 rewetting cycles increased C_m (55% increase in average) while decreased N_m (22% decrease
285 in average) with respect to the constant soil moisture treatment (Fig. 2). Percent changes of
286 C_m and N_m were not significantly different between seasons considering both ecotypes

287 together ($\chi^2 = 1.92$, $P = 0.166$ and $\chi^2 = 0.66$, $P = 0.417$, respectively). The temporal dynamics
288 of C mineralization in each water regime were similar for all soil samples regardless of the
289 ecotype, defoliation degree and season (Fig. 3a). Carbon mineralization boosted in each
290 rewetting event respect to the constant soil moisture regime (Fig. 3a), with R_{Hpulse} mean values
291 of 27.0, 9.9 and 5.7 mg C-CO₂ kg soil⁻¹ h⁻¹ (Fig. 4; Table S1).

292

293 *3.3 Effect of defoliation on the response of potential C and N mineralization rates to different* 294 *water regimes (HOD short-term effects)*

295 Soil samples collected in the holm oak ecotype showed significant changes in C_m with
296 water regime only in summer ($P < 0.05$), with no differences among defoliation degrees in
297 any season (Fig. 2). Considering each water regime separately, we found a significant and
298 negative effect of HOD on C_m of soil samples collected in summer and subjected to the water
299 cycles regime (Table 3). Soil samples collected under affected and dead trees showed a trend
300 to lower values of C_m compared to soils collected under healthy trees (30% decrease), with
301 significant differences ($P < 0.01$) one day after the first rewetting (Fig. 3b). Soil samples
302 collected under affected and dead trees also showed values of R_{Hpulse} for the second rewetting
303 event marginally lower than those of soils collected under healthy trees ($P = 0.06$; Table S1).
304 This negative effect of HOD was only marginally significant when we expressed C_m on a C
305 basis (Table S2). In the case of N_m , we found significant negative effects of water regime (P
306 < 0.05) for soils collected under affected and dead trees in both seasons, but never for samples
307 collected under healthy trees (Fig. 2). Considering each water regime separately, HOD did
308 not significantly affect N_m (Table 3).

309

310 *3.4 Effect of ecotype on the response of potential C and N mineralization rates to different*
311 *water regimes (HOD long-term effects)*

312 Potential C and N mineralization rates were always significantly higher ($P < 0.001$)
313 in the holm oak ecotype than in the grassland ecotype regardless of the water regime, season
314 and defoliation degree (Tables 2 and 3). These ecotype effects disappeared when we
315 expressed these variables on a C basis (Table S2).

316 Potential C and N mineralization rates of the grassland ecotype responded in the same
317 direction to the water regime change than those of the holm oak ecotype (with increased C
318 mineralization and decreased N mineralization) with significant ($P < 0.05$ in all cases) percent
319 changes regardless of the season and the defoliation degree (Figure 2). We found a significant
320 ecotype x water regime interaction ($P < 0.05$) in the C_m and N_m of soil samples collected in
321 summer (Table 2), with an average increase in C_m higher in the holm oak (71%) than in the
322 grassland (53%) ecotype, and an average decrease in N_m lower in the holm oak (16%) than
323 in the grassland ecotype (26%).

324

325 **4. Discussion**

326 *4.1 Response of potential C and N mineralization to changes in soil water regime*

327 The three rewetting events triggered rapid increases in microbial respiration within
328 the first 24 hours. Not surprisingly, the CO_2 pulses were progressively lower and slightly
329 shorter throughout the consecutive rewetting events, which can be largely explained by
330 substrate depletion as incubation progresses (Song et al., 2017). Rainfall pulses wet the soil
331 surface, making labile C and dead microbial biomass accumulated over the dry period
332 available for microbial decomposition (Blazewicz et al., 2014; Rey et al., 2017, 2005) and
333 producing changes in soil microbial communities (Barnard et al., 2013; Evans and

334 Wallenstein, 2014). Additionally, water physical CO₂ displacement from soil pore spaces
335 may also contribute to soil CO₂ pulses in the first hours upon rewetting (Rey et al., 2017).
336 Independently of the mechanisms behind the observed CO₂ pulses, our results corroborated
337 the rapid and strong response of semiarid soils to even small rainfall pulses after a drought
338 event (e.g. Rey et al., 2017; Song et al., 2017; Wang et al., 2016). This rapid CO₂ pulse after
339 a dry soil is rewetted (Birch effect) is of great interest due to its important implications for
340 soil C stocks (Blazewicz et al., 2014; Borken and Matzner, 2009), ecosystem C balances (Ma
341 et al., 2012) and global warming (Jarvis et al., 2007). This is particularly true in
342 Mediterranean and semiarid ecosystems where water availability is the main limiting factor
343 of ecosystem functioning (e.g. Jarvis et al., 2007; Rey et al., 2017; Song et al., 2017).

344 In our study, the observed decrease in N mineralization with the drying-rewetting
345 cycles respect to the constant moisture treatment disagreed with results from previous
346 laboratory studies (see Borken and Matzner, 2009). While those studies used extended
347 wetting and extreme and short drying periods, we simulated the natural Mediterranean
348 climatic conditions by using short and small rewetting events between relatively long drying
349 periods. The different length and intensity of the drying and rewetting events could largely
350 explain these contrasting results, as other studies using drying-rewetting cycles that simulated
351 natural conditions also found decreases in net N mineralization (Morillas et al., 2015).
352 Decreased N mineralization rates might be explained by substrate and/or water availability
353 limitation, changes in soil microbial community composition and/or activity (Stark and
354 Firestone, 1995), higher N immobilization (Gallardo et al., 2009) and increased N
355 denitrification (Morillas et al., 2015) during the drying-rewetting cycles regime. The HOD-
356 driven decrease in soil NH₄⁺-N in summer (Table 1), consistent with past research in the
357 same area (Rodríguez et al., 2017), supports the existence of a substrate-induced limitation

358 of nitrification. We did not register a significant effect of the water regimes on microbial
359 biomass (substrate-induced respiration, Table S3), but the drying-rewetting cycles could have
360 favored a soil microbial community with higher requirements of N and therefore lower C:N
361 ratios (Mouginot et al., 2014). Alternatively, whilst it is unlikely that our soils experienced
362 the anaerobic conditions traditionally considered necessary for denitrification, recent studies
363 have suggested that denitrification can be relevant even at high soil O₂ concentrations (Morse
364 et al., 2015a, 2015b). Our study does not allow us to confirm which of these mechanisms
365 drives the observed decrease in N mineralization. However, it clearly shows the potential
366 negative effect of increasing drying-rewetting cycles on the cycling and availability of these
367 nutrient, particularly as drought periods between erratic rainfall events are expected to be
368 more frequent and intense in Mediterranean forests due to climate change.

369 Overall, the drying-rewetting cycles treatment led to a 55% increase in the potential
370 C mineralization rate but a 22% decrease in the potential N mineralization rate compared
371 with the constant moisture treatment. These results confirmed our hypothesis about potential
372 C and N mineralization rates responding differently to changes in soil water regimes. More
373 importantly, these results support recent studies suggesting a different sensitivity and
374 response of C- and N- cycling to environmental changes (Durán et al., 2013; Morillas et al.,
375 2015, 2017; Rodríguez et al., 2017; Tan and Wang, 2016). A different response in C and N
376 mineralization could eventually lead to a decoupling of the C and N cycles, with the
377 subsequent major effects on ecosystem function, such as asynchronies in N supply and
378 demand or a decrease in the capacity of forest to sequester C (Fernández-Martínez et al.,
379 2014; Finzi et al., 2011; Rodríguez et al., 2014).

380

381 4.2 Short- and long-term effects of HOD on the response of potential C and N mineralization
382 rates to different soil water regimes

383 In our study, whereas differences among defoliation degrees in soils collected under
384 holm oak trees indicate short-term (years) responses to holm oak decline, differences between
385 ecotypes (holm oak vs. grassland) might be indicative of longer-term (decades/centuries)
386 changes in ecosystem functioning. On the short term, HOD did not significantly affect the
387 response of soil C mineralization to changes in the water regime, but we did find a significant
388 effect of defoliation degree on the C_m of summer soil samples subjected to the cycles
389 treatment. Further, we observed a negative, long-term HOD effect on the response of soil C
390 mineralization to changes in water regime, as soils collected in summer under the grassland
391 ecotype showed lower increases in the C_m compared to those collected under the holm oak
392 ecotype. Such lower response of soils from the grassland than from the holm oak ecotype
393 could be largely explained by the lower soil total C content in the grassland samples (Table
394 1). Soils collected below different defoliation degrees did not show significant differences in
395 soil C content. However, the fact that the differences observed in C_m among defoliation
396 degrees were only marginal when we expressed this variable on a C basis points out both soil
397 C content and quality as possible drivers of those differences.

398 Microbial communities less diverse and with different historical water regimes may
399 have also contributed to curtail the response of C mineralization to the drying-rewetting
400 events (Wang et al., 2016). For instance, soil bacterial communities showed decreasing
401 functional richness with HOD both at the short- and at the long-term (Table 1). In a previous
402 study carried out in the same site, Rodríguez et al. (2017) found a HOD-driven ecotype
403 convergence in the soil microbial community tightly linked to a cascade effect where *Q. ilex*
404 is being replaced by understory species (savannization process). Thus, soil microbial

405 communities under affected and dead holm oaks, as well as under the grassland, are likely to
406 be similar, and therefore have a similar response to environmental changes. Further, the
407 decreased canopy cover could have selected soil microbial communities better adapted to
408 both drying and wetting stress (Edburg et al., 2012), thus minimizing their response to them
409 (Borken and Matzner, 2009).

410 Regarding potential N mineralization rates, only soil samples collected under affected
411 and dead holm oaks showed a significant decrease in the cycles regime compared to the
412 constant moisture treatment (Figure 2). Further, summer soil samples collected under the
413 grassland ecotype showed a decrease in N mineralization 38% higher than those collected
414 under the holm oak ecotype. Thus, HOD apparently modulated the response to changes in
415 water regime at both the short- and the long-term. The higher sensitivity of N mineralization
416 rates to water regime changes under a HOD scenery might be related to the lower functional
417 richness and differences in soil microbial composition above discussed. More importantly,
418 these results suggest that the interactive effect of climate change and forest die-off is likely
419 to exacerbate the climate change-driven decrease in N availability discussed in the previous
420 section.

421 The differences in the direction and intensity of the response of potential C and N
422 mineralization to the interactive effects of water regime and holm oak decline supports our
423 first hypothesis, and evidence a higher probability of C and N decoupling with the interaction
424 of both environmental disturbances. All these results stress the importance of studying the
425 interactive effects of multiple environmental changes in the ecosystem functioning
426 (Matesanz et al., 2009).

427

428 4.3 Season as modulator of the response of potential C and N mineralization rates to changes
429 in the water regime and and HOD

430 Our results confirmed the role of season as modulator of the microbial functioning
431 response to changes in the water regime and holm oak decline, particularly for the holm oak
432 ecotype and the C mineralization process. Summer was the season when drying-rewetting-
433 driven increased soil CO₂ emissions under holm oaks were significant. Similarly, HOD short-
434 term effects on the response of potential C mineralization and long-term effects on the
435 response of potential C and N mineralization to drying-rewetting cycles were more noticeable
436 in summer. Increased C mineralization due to rewetting after summer drought has been
437 widely observed (Borken and Matzner, 2009; Song et al., 2017). However, to our knowledge,
438 this is the first study exploring how forest die-off affects the response of soil microbial
439 functioning to changes in water regime, as well as the temporal variability of that response.
440 The higher impact of HOD on the response of potential C and N mineralization rates to
441 drying-rewetting cycles in summer agrees with the results from a study carried out in
442 Mediterranean *Quercus suber* forests (Ávila et al., 2016). That study explains the lower
443 likelihood to detect differences in C and nutrient cycling between healthy and declining or
444 dead trees in these ecosystems during spring due to the homogenizing effect of grass (Tang
445 and Baldocchi, 2005). However, we here show that, at the short-term, potential N
446 mineralization responded consistently and independently of the season to changes in water
447 regime and the HOD and water regime interaction. This suggest a higher sensitivity of the N
448 mineralization process to environmental disturbances and strengthens the need for further
449 research on the largely unknown response of N cycle related-processes to global change
450 scenarios.

451 The lack of seasonal differences in the response of potential C and N mineralization
452 to water regime changes in the grassland ecotype also suggests a more extensive sensitivity
453 of its soil microbial community to climate change. The projected more frequent, hotter and
454 longer drought events in these forests structured by a single, strongly dominant tree species
455 could drive to a scenario where trees would be replaced by the smaller understory species
456 (Saura-Mas et al. 2014; Ibáñez et al. 2015). Herein, we show that in such savannization
457 scenario, this type of Mediterranean ecosystems would be less capable to sequester (Table
458 1), and therefore, mineralize C and N in soil (Table 2). More importantly, this savannization
459 of the Mediterranean woodlands would likely make the system more vulnerable to projected
460 changes in water regimes, compromising important ecosystem functions, such as soil organic
461 matter mineralization and nutrients turnover.

462

463 **Conclusions**

464 Our study add new evidence on the direction in which Mediterranean forest soils will
465 respond to forecasted changes in rainfall regimes, revealing asymmetric responses in C and
466 N mineralization. As soil drying-rewetting cycles are predicted to increase in frequency and
467 intensity in the next decades due to climate change, such asymmetry is likely to result not
468 only in important soil C losses but also in significant decreases in N availability for plants
469 and microorganisms, with important implications for ecosystem functioning and services.
470 Further, our study provides new and valuable insights on the hitherto unexplored interactive
471 effects of drying-rewetting cycles and forest die-off on soil C and N mineralization. We show
472 that tree decline and mortality might enhance the probability of C and N decoupling at both
473 short- and long-terms, by minimizing the response of C mineralization but accentuating the
474 response of N mineralization to drying-rewetting cycles, with a higher sensitivity of the N

475 than of the C mineralization during most of the year. Further research is needed however to
476 gain a better understanding of the mechanism behind these asymmetric responses of C and
477 N mineralization, as well as of the implications of these two increasing environmental
478 disturbances on the microbial functioning of Mediterranean forests.

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Tables

Table 1. Main biogeochemical ($n = 6$) and bacterial functional alpha-diversity ($n = 5$) variables. Values represent the mean (± 1 SE). Statistically significant effects of defoliation (P_d) and ecotype (P_e) are represented by bold P values. Different letters represent significant differences among defoliation degrees in each ecotype ($P < 0.05$, ANOVA). Underlined values denote significant differences between ecotypes for the respective defoliation degree and season ($P < 0.05$). TC = total carbon; TN = total nitrogen; SIR = substrate-induced respiration; H'_{bact} = soil bacterial functional Shannon index; S'_{bact} = soil bacterial functional richness, Sp = spring; Su = summer.

		Holm oak ecotype			Grassland ecotype			Mixed models	
		Healthy	Affected	Dead	Healthy	Affected	Dead	P_d	P_e
TC	Sp	<u>3.06 (0.72)</u>	<u>2.34 (0.28)</u>	<u>2.60 (0.31)</u>	<u>1.04 (0.08)</u>	<u>1.38 (0.21)</u>	<u>1.33 (0.18)</u>	0.925	<0.001
TN	Sp	<u>0.24 (0.04)</u>	<u>0.19 (0.02)</u>	<u>0.20 (0.02)</u>	<u>0.11 (0.01)</u>	<u>0.10 (0.02)</u>	<u>0.12 (0.01)</u>	0.365	<0.001
NH ₄ ⁺ -N	Sp	<u>5.28 (2.01)</u>	1.83 (0.90)	2.84 (0.85)	<u>0.10 (0.10)</u>	0.08 (0.08)	1.51 (1.36)	0.387	<0.001
	Su	<u>7.64 (1.41)a</u>	3.87 (0.70)ab	3.66 (0.71)b	<u>4.15 (2.22)</u>	2.68 (0.60)	4.09 (0.88)	0.293	0.014
NO ₃ ⁻ -N	Sp	<u>4.96 (0.73)</u>	<u>5.59 (0.57)</u>	4.24 (0.21)	<u>2.48 (0.59)</u>	<u>2.21 (0.57)</u>	2.39 (0.38)	0.605	<0.001
	Su	1.07 (0.22)	1.84 (0.23)	1.75 (0.33)	1.39 (0.22)	1.35 (0.12)	1.85 (0.33)	0.095	0.894
SIR	Sp	31.5 (9.28)	38.7 (4.73)	46.0 (16.0)	17.7 (4.62)	13.8 (2.39)	39.5 (8.37)	0.073	0.036
	Su	20.9 (6.67)	16.1 (1.86)	15.6 (2.77)	10.1 (2.32)	8.38 (1.43)	8.42 (1.24)	0.775	<0.001
H'_{bact}	Sp	<u>4.47 (0.05)</u>	<u>4.39 (0.06)</u>	4.47 (0.07)	<u>4.09 (0.09)</u>	<u>3.90 (0.12)</u>	4.28 (0.11)	0.027	<0.001
	Su	4.17 (0.05)	<u>4.07 (0.10)</u>	3.89 (0.06)	4.02 (0.10)	<u>3.68 (0.14)</u>	3.75 (0.09)	0.052	<0.001
S'_{bact}	Sp	27.8 (0.58)	27.2 (0.80)	27.8 (0.58)	24.0 (1.52)	23.2 (1.46)	26.4 (1.17)	0.212	<0.001
	Su	25.0 (0.77)a	23.0 (1.55)ab	19.6 (1.63)b	21.8 (2.11)	17.8 (2.13)	16.8 (2.08)	0.042	0.001

TC and TN are expressed in %; NH₄⁺-N and NO₃⁻-N are expressed in mg N kg soil⁻¹; SIR is expressed in mg C kg soil⁻¹ h⁻¹

Table 2. Statistical results of mixed models to test the effect of defoliation degree, ecotype and water regime on the potential C and N mineralization rates (C_m and N_m , respectively) of soil samples collected in spring and summer. Statistically significant effects of defoliation (D), ecotype (E) and water regime (WR), as well as significant interactions among factors, are represented by bold P values.

Mixed models	Spring			Summer		
	χ^2	df	P	χ^2	df	P
C_m						
D	1.169	2	0.558	3.532	2	0.171
E	21.466	1	< 0.001	14.490	1	< 0.001
WR	17.437	1	< 0.001	100.766	1	< 0.001
D x E	1.565	2	0.457	1.465	2	0.481
D x WR	3.289	2	0.193	2.638	2	0.267
E x WR	0.566	1	0.452	6.279	1	0.012
D x E x WR	3.290	2	0.193	1.414	2	0.493
N_m						
D	1.504	2	0.472	0.448	2	0.799
E	114.411	1	< 0.001	23.097	1	< 0.001
WR	61.164	1	< 0.001	59.935	1	< 0.001
D x E	3.808	2	0.149	0.479	2	0.787
D x WR	5.126	2	0.077	1.576	2	0.455
E x WR	3.268	1	0.071	4.334	1	0.037
D x E x WR	2.681	2	0.262	6.018	2	0.049

Variables expressed as mg C-CO₂ or N kg⁻¹ soil day⁻¹

Table 3. Means (± 1 SE) of potential C and N mineralization rates (C_m and N_m , respectively) of soil samples collected in spring and summer under the holm oak (H) and the grassland (G) ecotype of healthy, affected and dead trees and subject to the two water regimes (constant moisture vs. drying-rewetting cycles). Statistically significant effects of defoliation (P_d) and ecotype (P_e) are represented by bold P values. Underlined values denote significant differences between ecotypes for the respective defoliation degree and water regime ($P < 0.05$).

		Constant moisture			Mixed models		Drying-rewetting cycles			Mixed models	
		Healthy	Affected	Dead	P_d	P_e	Healthy	Affected	Dead	P_d	P_e
Spring											
C_m	H	<u>93.2 (26.6)</u>	52.8 (5.33)	<u>82.6 (22.5)</u>	0.491	<0.001	<u>96.4 (22.1)</u>	72.8 (13.2)	<u>92.2 (18.2)</u>	0.799	<0.001
	G	<u>34.3 (5.43)</u>	37.1 (8.46)	<u>21.2 (4.78)</u>			<u>39.3 (4.66)</u>	40.1 (5.83)	<u>39.2 (5.86)</u>		
N_m	H	1.52 (0.09)	1.87 (0.15)	1.84 (0.20)	0.403	<0.001	<u>1.33 (0.10)</u>	<u>1.34 (0.07)</u>	<u>1.35 (0.14)</u>	0.535	<0.001
	G	0.87 (0.10)	0.70 (0.06)	1.05 (0.21)			<u>0.66 (0.07)</u>	<u>0.48 (0.05)</u>	<u>0.74 (0.11)</u>		
Summer											
C_m	H	<u>35.7 (7.10)</u>	<u>23.6 (7.32)</u>	<u>23.6 (5.79)</u>	0.189	0.009	<u>53.3 (3.58)</u>	34.2 (6.79)	36.0 (5.11)	0.034	<0.001
	G	<u>21.1 (5.45)</u>	<u>15.1 (1.29)</u>	<u>15.2 (2.14)</u>			<u>29.6 (6.45)</u>	22.0 (2.55)	24.2 (3.31)		
N_m	H	1.73 (0.24)	<u>1.82 (0.29)</u>	1.74 (0.23)	0.637	<0.001	1.52 (0.16)	1.33 (0.11)	<u>1.46 (0.18)</u>	0.541	<0.001
	G	1.31 (0.32)	<u>0.91 (0.06)</u>	1.30 (0.18)			0.97 (0.24)	0.77 (0.08)	<u>0.84 (0.15)</u>		

$n = 6$; variables expressed as mg C-CO₂ or N kg⁻¹ soil day⁻¹; Values of constant moisture regime obtained from Rodríguez et al. (2017).

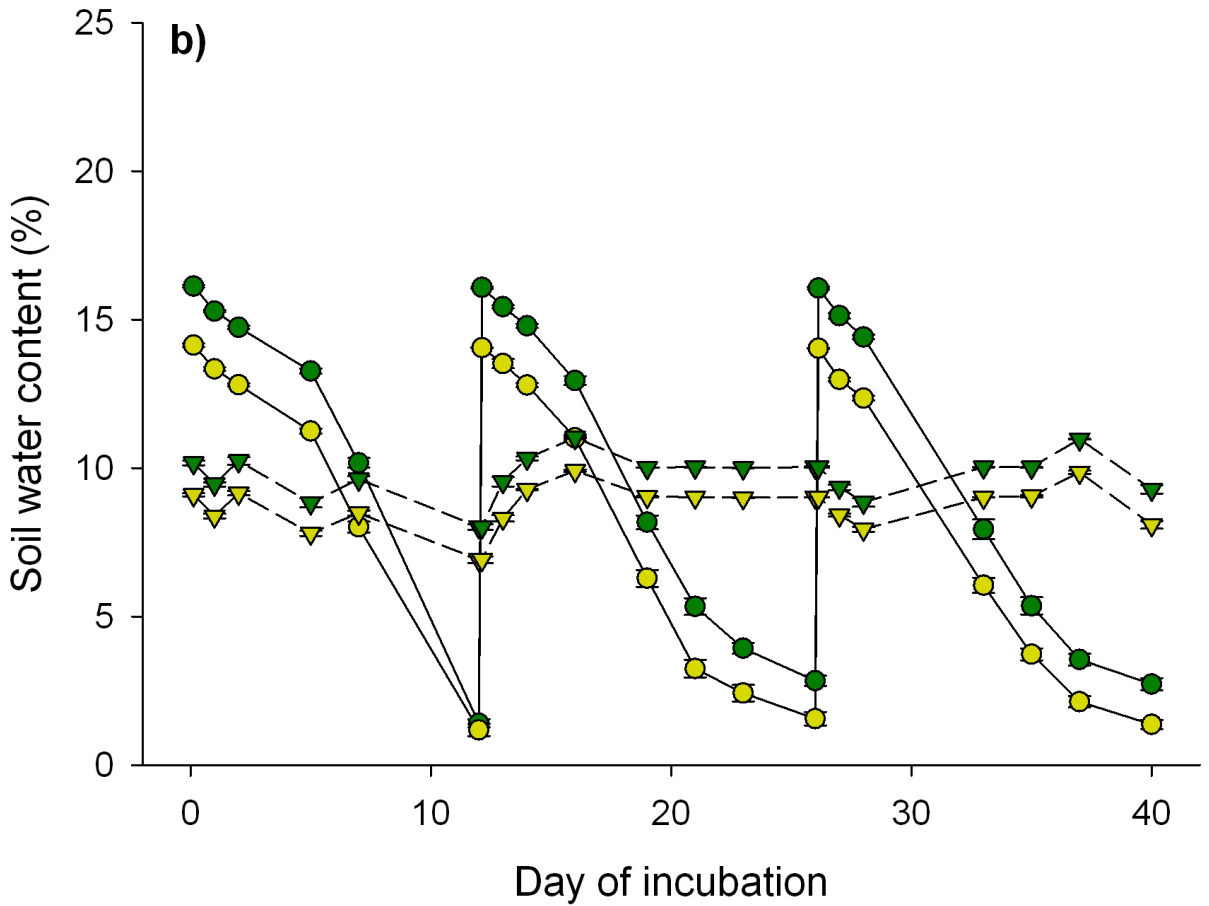
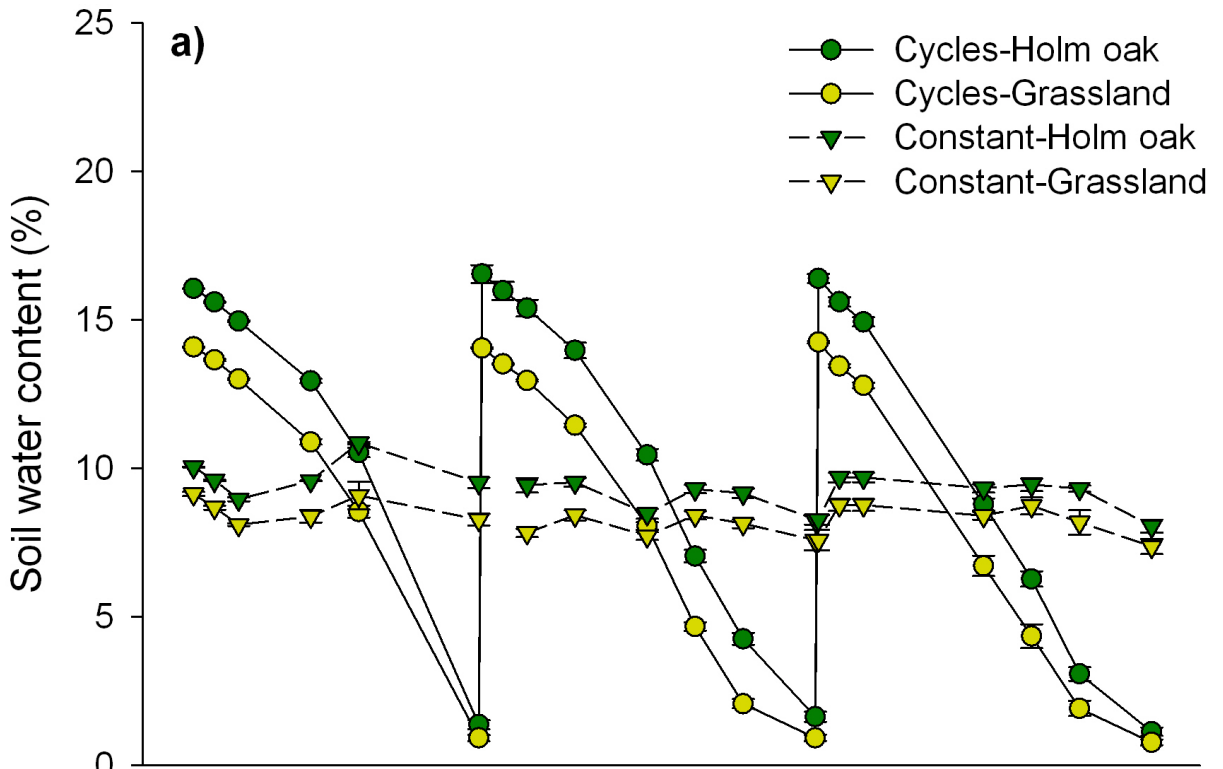
Figure captions

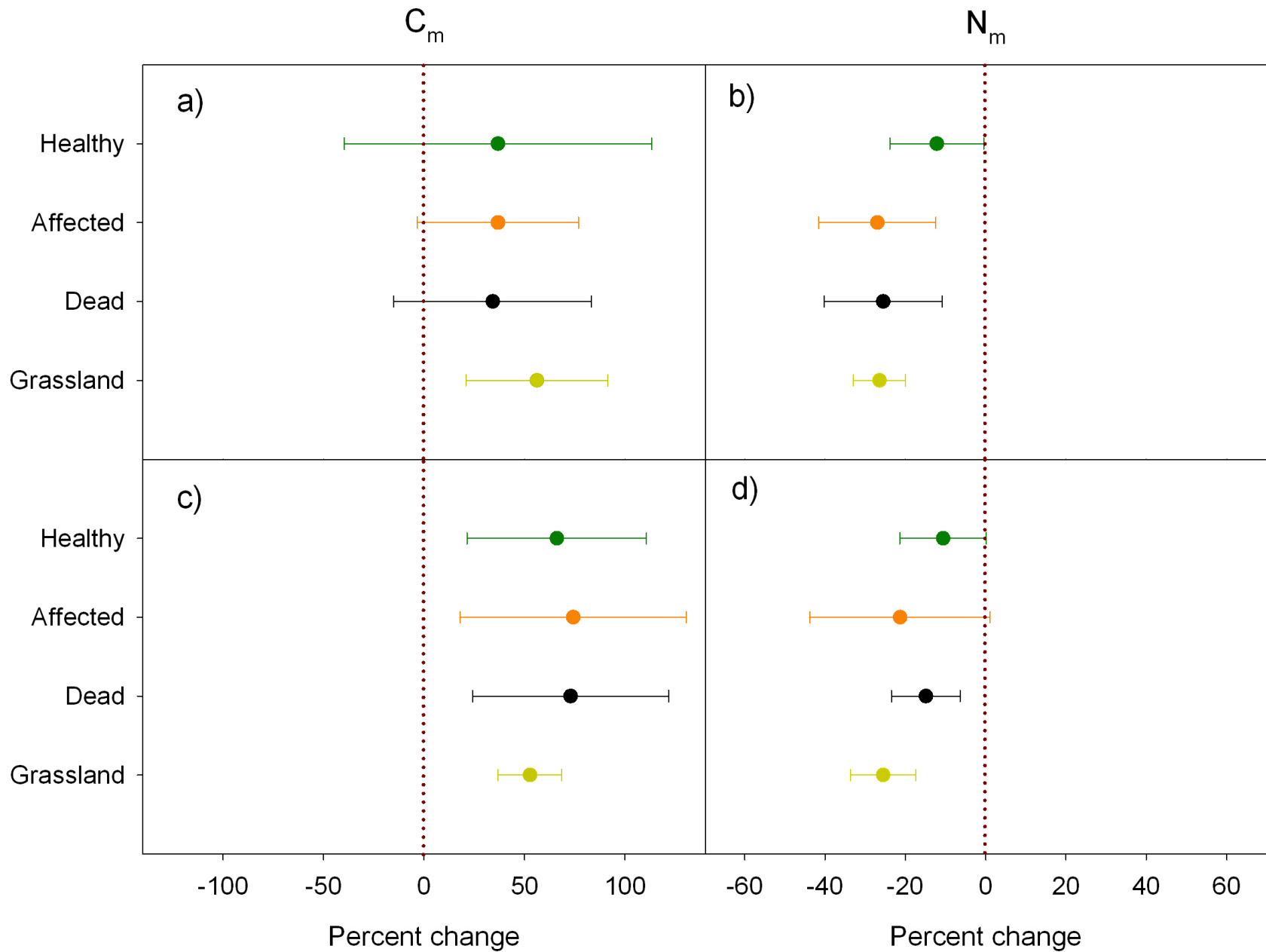
Figure 1. Dynamic of soil moisture of samples collected under the holm oak and the grassland ecotype both in spring (a) and summer (b), and subjected to the drying-rewetting cycles and the constant moisture regime. Symbols and error bars represent means ($n = 18$) and standard errors (1SE) of the mean, respectively.

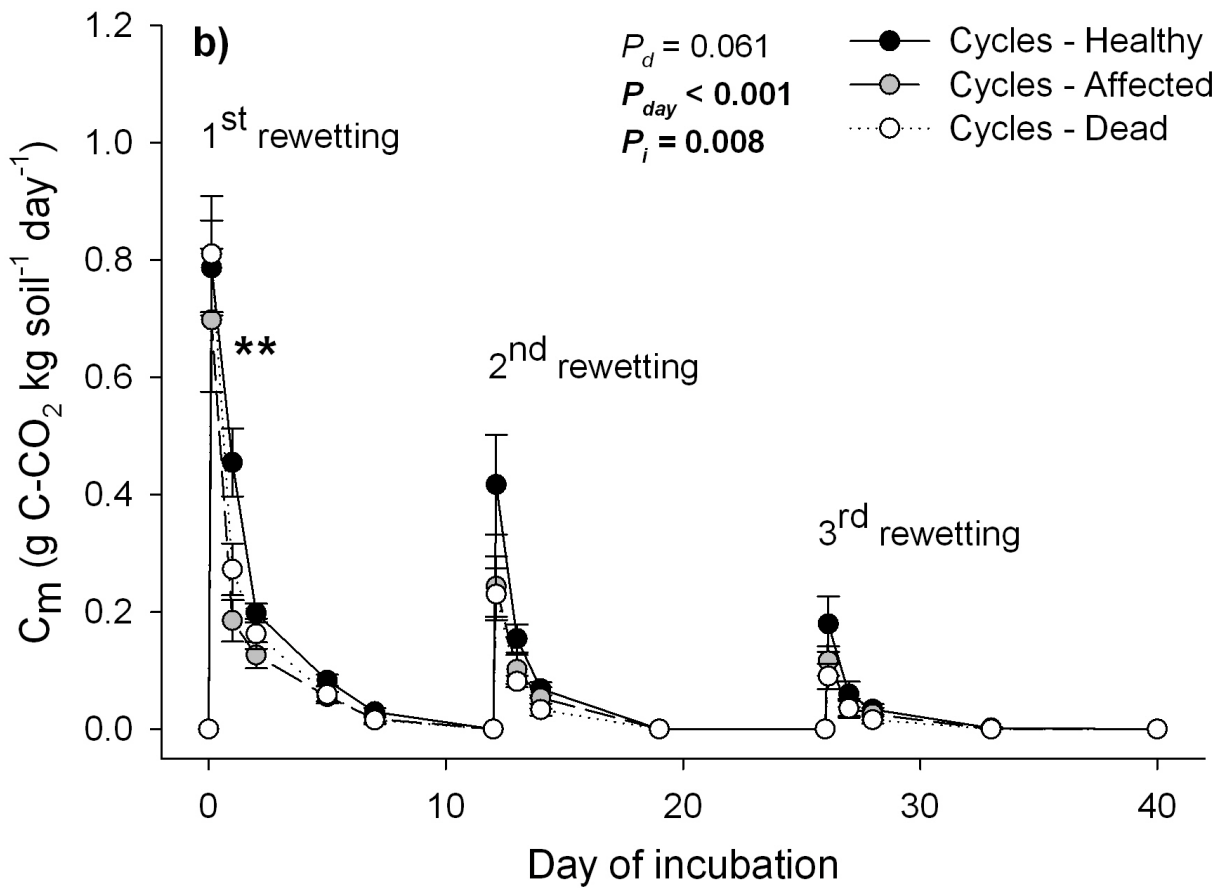
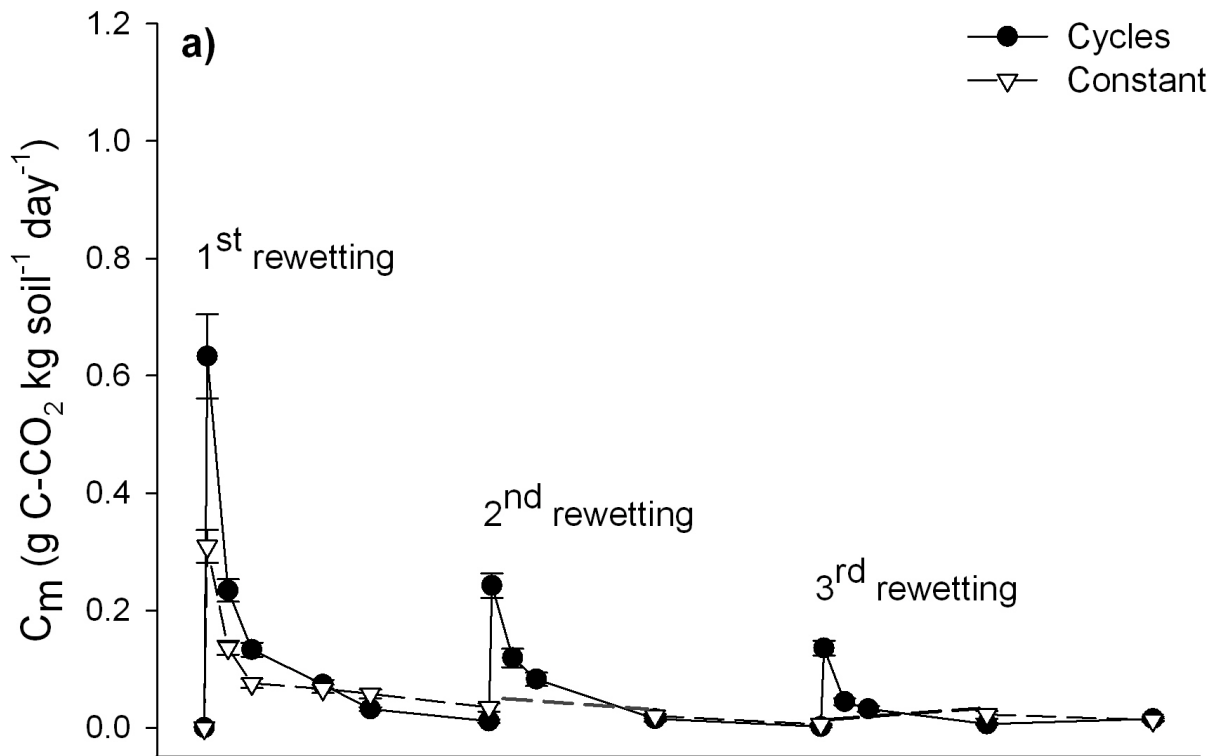
Figure 2. Percent change in potential C and N mineralization rates (C_m and N_m , respectively) between the drying-rewetting cycles and the constant soil moisture regime treatments. Dots are the mean of soil samples collected under healthy, affected and dead trees and under the grassland ecotype both in spring (a-b) and summer (c-d) and subject to the 40-day laboratory incubation ($n = 6$). Error bars indicate the 95% confidence interval. Positive values indicate that drying-rewetting cycles had a positive effect compared to the constant soil moisture treatment, negative values indicate the opposite.

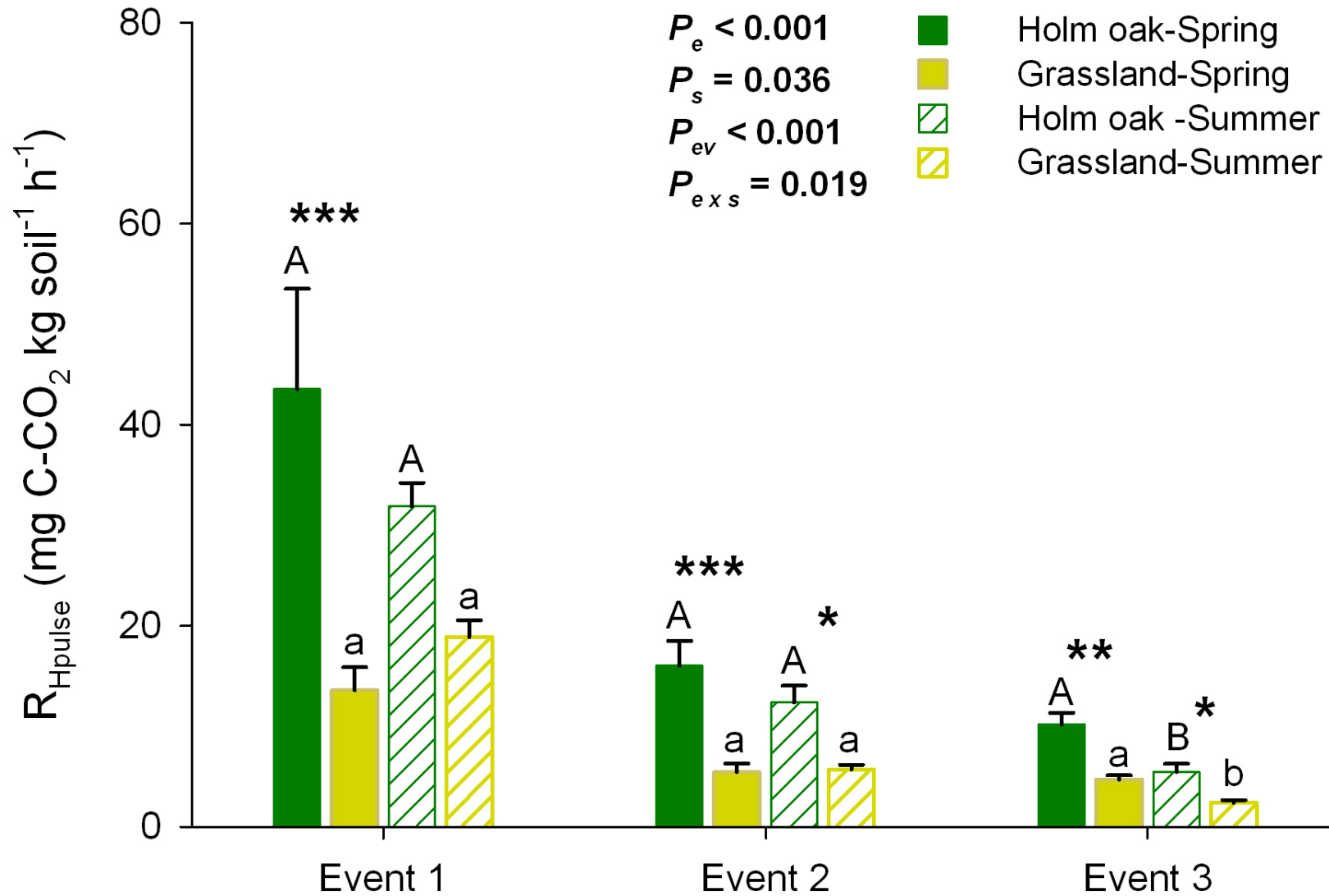
Figure 3. Temporal dynamics of potential C mineralization rate of: a) all soil samples together ($n = 36$) for each one of the water regimes (drying-rewetting cycles vs. constant soil moisture); and b) samples collected in summer under healthy, affected and dead trees (holm oak ecotype) and subject to the drying-rewetting cycles regime ($n = 6$). Symbols and error bars represent means and standard errors (1SE), respectively. Significant differences among defoliation degrees for each one of the incubation days individually are denoted by: ** = $P < 0.01$. P_d , P_{day} and P_i show the significant level of defoliation degree, incubation day and the interaction of both, respectively (mixed models).

Figure 4. Pulse effect of the three rewetting events on microbial respiration (R_{Hpulse}) of soils samples collected in spring (plain bars) and summer (stripped bars) under the holm oak (green bars) and the grassland (yellow bars) ecotypes. Bars and error bars represent means ($n = 18$) and standard errors (1SE), respectively. Different letters within each ecotype represent significant differences between seasons ($P < 0.05$). Asterisks indicate significant differences among ecotypes (* = $P < 0.05$; ** $P < 0.01$; *** = $P < 0.001$). P_e , P_s , P_{ev} and $P_{e \times s}$ show the significant level of ecotype, season and event, and the interaction between ecotype and season, respectively (mixed models).









Supplementary material

Table S1. Means (1SE) of the pulse effect (R_{Hpulse}) of each rewetting event in the soils samples collected in spring (Sp) and summer (Su) under the holm oak and the grassland ecotypes of healthy, affected and dead trees. Statistically significant effects of defoliation (P_d) and ecotype (P_e) are represented by bold P values. Underlined values denote significant differences between ecotypes for the respective defoliation degree and season ($P < 0.05$).

		Holm oak			Grassland			Mixed models	
		Healthy	Affected	Dead	Healthy	Affected	Dead	P_d	P_e
R_{Hpulse1}	Sp	44.1 (14.7)	39.2 (16.7)	47.3 (22.7)	11.8 (2.08)	18.8 (5.68)	10.1 (2.93)	0.724	< 0.001
	Su	32.8 (3.38)	<u>29.1 (5.09)</u>	<u>33.7 (4.12)</u>	21.5 (4.38)	<u>15.6 (0.42)</u>	<u>19.5 (2.42)</u>	0.269	< 0.001
R_{Hpulse2}	Sp	<u>18.2 (5.73)</u>	11.7 (3.29)	<u>18.2 (3.56)</u>	<u>4.48 (1.11)</u>	4.62 (1.15)	<u>7.15 (2.03)</u>	0.405	< 0.001
	Su	<u>17.4 (3.55)</u>	10.1 (2.14)	9.58 (1.84)	<u>6.40 (0.73)</u>	5.18 (0.74)	5.47 (1.03)	0.058	< 0.001
R_{Hpulse3}	Sp	<u>10.7 (2.86)</u>	<u>10.1 (1.65)</u>	9.73 (1.57)	<u>4.50 (0.76)</u>	<u>4.63 (0.91)</u>	4.88 (0.73)	0.999	< 0.001
	Su	<u>7.47 (1.96)</u>	4.83 (1.05)	3.99 (0.87)	<u>2.58 (0.56)</u>	2.20 (0.52)	2.37 (0.31)	0.499	< 0.001

$n = 6$; Variable expressed as mg C-CO₂ kg soil⁻¹ h⁻¹

Table S2. Means (1SE) of potential C and N mineralization rates (C_m and N_m , respectively) expressed on a carbon basis of soil samples collected in spring and summer under the holm oak (H) and the grassland (G) ecotype of healthy, affected and dead trees and subjected to the drying-rewetting cycles regime. P_d , P_e and P_i show the significant level of the defoliation degree, the ecotype and the interaction of both, respectively (mixed models).

		Healthy	Affected	Dead	Mixed models		
					P_d	P_e	P_i
Spring							
C_m	H	3.17 (0.22)	3.03 (0.18)	3.43 (0.44)	0.615	0.711	0.327
	G	3.77 (0.34)	3.23 (0.57)	2.95 (0.29)			
N_m	H	51.3 (6.71)	59.7 (5.29)	53.9 (5.55)	0.590	0.941	0.063
	G	66.4 (11.4)	41.5 (5.71)	56.6 (5.72)			
Summer							
C_m	H	2.05 (0.29)	1.63 (0.47)	1.39 (0.12)	0.197	0.070	0.919
	G	3.13 (0.91)	1.90 (0.45)	1.95 (0.33)			
N_m	H	59.4 (11.2)	59.3 (5.37)	59.4 (8.01)	0.754	0.261	0.437
	G	100.3 (32.5)	59.7 (8.54)	73.1 (21.2)			

$n = 6$; variables expressed as g C-CO₂ or mg N kg⁻¹ C day⁻¹

Table S3. Means (1SE) of the percent change (%) of substrate-induced respiration (SIR) values during the incubation of soil samples collected in spring (Sp) and summer (Su) under the holm oak (H) and the grassland (G) ecotype of healthy, affected and dead trees and subjected to the two water regimes. P_d , P_{wr} and P_i show the significant level of the defoliation degree, the water regime and the interaction of both, respectively (mixed models).

		Drying-rewetting cycles			Constant moisture			Mixed models		
		Healthy	Affected	Dead	Healthy	Affected	Dead	P_d	P_{wr}	P_i
H	Sp	75.5 (111.5)	-58.9 (12.1)	15.2 (59.4)	33.2 (83.1)	-60.2 (18.9)	55.5 (115.2)	0.309	0.986	0.870
	Su	-66.9 (8.7)	-71.8 (4.5)	-67.8 (4.9)	-64.6 (8.7)	-73.4 (4.0)	-53.6 (17.7)	0.451	0.519	0.682
G	Sp	-31.4 (26.5)	-25.7 (13.3)	-66.5 (14.3)	-20.9 (25.9)	-53.8 (7.4)	-70.7 (10.3)	0.069	0.622	0.559
	Su	-61.1 (5.0)	-33.5 (29.2)	-65.8 (7.2)	-64.9 (8.7)	-51.5 (29.0)	-79.1 (8.2)	0.246	0.429	0.922

Percent change = $((SIR_I - SIR_F) / SIR_I) * 100$; where SIR_I is the initial value (before) and SIR_F is the final value (after incubation).