1   **Strong resilience of soil respiration components to drought-induced die-off**

2   **resulting in forest secondary succession**

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4   Running head: Soil respiration partitioning fluxes

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19   *Globally increasing drought-induced forest die-off and its associated vegetation shifts*

20   *may have direct impacts on soil respiration. Here, we found that soil respiration and its*

21   *autotrophic and heterotrophic components remained unaffected 3-11 years following*

22   *drought-induced Scots pine die-off. Despite this post-disturbance functional resilience,*

23   *the replacement by holm oak was associated with a strong reduction in the*

24   *heterotrophic respiration component, producing an important drop in total soil*

25   *respiration.*

26

27   Author contributions: JB, JCY and FLL conceived and designed the experiment; JB,

28   JCY and RP performed the experiment; JB, JCY, IJ and RP analysed the data; JB wrote

29   the paper and all authors edited the manuscript.

30

Abstract

How forests cope with drought-induced perturbations and how the dependence of soil respiration on environmental and biological drivers is affected in a warming and drying context are becoming key questions.

The aims were to determine whether drought-induced die-off and forest succession were reflected in soil respiration and its components and to determine the influence of climate on the soil respiration components.

We used the mesh exclusion method to study seasonal variations soil respiration (R_S) and its components: heterotrophic (R_H) and autotrophic (R_A) (further split in fine root [R_R] and mycorrhizal respiration [R_M]) in a mixed Mediterranean forest where Scots pine (*Pinus sylvestris* L.) is undergoing a drought-induced die-off and is being replaced by holm oak (*Quercus ilex* L.).

Drought-induced pines die-off was not reflected in R_S nor in its components which denotes a high functional resilience of the plant-and-soil system to pines die-off. However, the succession from Scots pines to holm oaks resulted in a reduction of R_H and thus in an important decrease of total respiration (R_S was 36% lower in holm oaks than in non-defoliated pines). Furthermore, R_S and all its components were strongly regulated by SWC-and-temperature interaction.

Since Scots pine die-off and *Quercus* species colonization seems to be widely occurring on the driest limit of the Scots pine distribution, the functional resilience of the soil system over die-off and the decrease of R_S from Scots pine to holm oak could have direct consequences on the carbon balance of these ecosystems.

1. Introduction

Drought-induced forest die-off episodes are being increasingly reported globally (Allen et al. 2010), raising concerns on their possible association with recent global warming, at least in some parts of the globe (Carnicer et al. 2011). Increased tree defoliation and mortality rates have been related to chronic and episodic drought in the Mediterranean region (Peñuelas et al. 2001; Carnicer et al. 2011), where the projected increase in the frequency and intensity of droughts (IPCC, 2013) may enhance the risk of extensive forest die-off. Widespread drought-induced tree mortality can have dramatic effects on forest carbon cycling (Reichstein et al. 2013) which may differ from those reported for other disturbances such as fire or pest outbreaks (Anderegg et al. 2013; Borkhuu et al. 2015). Forest ecosystems store over two thirds of the carbon in their soils (Dixon et al.

65   1994), but the impact of drought-induced tree mortality on soil carbon fluxes and their

66   post-disturbance dynamics remains poorly understood (Allen et al. 2015).

67   The trajectory of ecosystem C cycling in stands where the dominant overstorey species

68   has been severely affected by a die-off event is complex in space and time (Edburg et al.

69   2012; Reed et al. 2014; Borkhuu et al. 2015). At subdecadal timescales, the trend in

70   forest C uptake largely depends on disturbance intensity and the degree of overstorey

71   canopy loss, the spatial pattern of tree dieback (i.e. clustered, diffuse), the compensatory

72   responses by surviving dominant trees, and the response of understorey vegetation to

73   competition release (Amiro et al. 2010; Brown et al. 2010; Gough et al. 2013). With

74   regard to forest soil C losses, die-off episodes immediately curtail root and mycorrhizal

75   respiration and reduce exudate supply from roots to soil, but they also stimulate

76   decomposition of litter, roots and dead wood (Nave et al. 2011). Moreover, die-off

77   episodes may increase soil moisture (Redding et al. 2008), which could enhance soil

78   organic matter (SOM) decomposition rates in water limited ecosystems. Nevertheless,

79   our knowledge on post-dieback ecosystem (and soil) C fluxes is still incomplete,

80   because (1) it largely arises from studies where pests or wildfires, not drought, were the

81   main disturbance drivers and (2) it lacks a detailed understanding of the post-dieback

82   trajectories of soil respiration components (heterotrophic respiration, R_H; autotrophic

83   respiration R_A; fine roots respiration, R_R; mycorrhizal respiration, R_M) and their

84   responses to environmental drivers.

85   Drought-induced tree mortality episodes often result in a complex spatial pattern of

86   standing dead trees and partially defoliated individuals, together with apparently

87   unaffected trees. These episodes may cause vegetation shifts within years or decades if

88   adult mortality and recruitment of the dominant and most affected species are not

89   balanced through time (Lloret et al. 2012). Given that the substituting species will likely

90   be more drought-resistant, such species replacement could have persistent effects on

91   both stand C uptake and release processes. The spatial distribution of soil CO_2 effluxes

92   under non-limiting soil moisture conditions is sensitive to local changes in vegetation

93   composition following tree mortality (Barba et al. 2013), but we do not know whether

94   the response of soil CO_2 effluxes to the main abiotic drivers (i.e. soil temperature and

95   moisture) differs along the stages of an ongoing vegetation shift. In Mediterranean

96   forests, the seasonal correlation between plant productivity and moisture-and-

97   temperature course hinders to determine the effect of these variables on R_S (Tedeschi et

98   al. 2006) because of the inherent seasonal variability of plant photosynthetic activity

99   and belowground C allocation (Reichstein et al. 2002; Keenan et al. 2009).

100   The variability of soil CO_2 efflux associated to the environmental drivers may also be

101   mediated by the differential metabolic response of microbes, roots and rhizosphere to

102   moisture and temperature changes (Uren 2000; Kuzyakov 2006; Moyano et al. 2010).

103   Changes in microbial community composition have been observed following drought-

104   induced forest die-off and succession (Curiel Yuste et al. 2012), but how these shifts in

105   microbial community composition modify the contribution of autotrophic and

106   heterotrophic respiration to total soil respiration remains poorly known.

107   Drought-induced dieback episodes, characterised by increased crown defoliation and

108   mortality rates, have been reported in several Scots pine (*Pinus sylvestris* L.)

109   populations in the northeast of the Iberian Peninsula (Martínez-Vilalta and Piñol 2002;

110   Galiano et al. 2010; Hereş et al. 2012). In particular, extreme drought events together

111   with a lack of forest management have caused several Scots pine die-off episodes at the

112   Prades Mountains in the 1990s and 2000s (Martínez-Vilalta and Piñol 2002; Hereş et al.

113   2012) and the associated holm oak (*Quercus ilex* L.) colonisation (Vilà-Cabrera et al.

114   2013). These processes have resulted in important changes not only at above-ground

115   component of the forest (Aguadé et al. 2015) but also at the below-ground part, altering

116   soil microbial diversity and structure (Curiel Yuste et al. 2012), litter decomposition and

117   nutrients cycling (Barba et al. 2015) and spatial variability of soil respiration (Barba et

118   al. 2013). In this study, we aim to quantify the effects of this Scots pine drought-related

119   die-off and the consequent holm oak colonisation on soil respiration and its

120   components, at seasonal and annual timescales. During one year, we measured the

121   seasonal variation of soil respiration and its heterotrophic and autotrophic (roots and

122   mycorrhiza) components, associated with the different stages of this drought-induced

123   die-off and species-replacement process: non-defoliated pines [NDP], partially

124   defoliated pines [DFP], dead pines [DP] and holm oaks [HO].

125   We hypothesized that: 1) Heterotrophic respiration would show a gradual increase

126   across this die-off gradient (from NPD to DFP and DP) and autotrophic respiration

127   would decrease because of reduced above-and-belowground plant activity in DFP and

128   DP. Given that holm oak is a more drought-tolerant species compared to Scots pine, for

129   this drought-exposed site we expected (2) higher soil respiration in holm oak and lower

130   sensitivity to decreasing soil moisture during summer drought compared to Scots pine.

131   Finally, we hypothesized that (3a) the spatial and temporal variation in heterotrophic

soil respiration would be mainly dependent on environmental variables (soil temperature and moisture), whereas these variables would explain little variability for autotrophic respiration. Since vegetation has the capacity to modify soil environmental conditions, we also hypothesized that (3b) the vegetation die-off and the ongoing species succession would largely influence soil CO_2 effluxes via modifications in environmental conditions such as SWC.

2. Materials and Methods

2.1. Study site

The study was conducted in a mixed forest in the Titllar Valley (Prades Mountains, NE Iberian Peninsula). The climate is Mediterranean, with a mean annual temperature of 11.2°C and precipitation of 664 mm (Ninyerola et al. 2007a; Ninyerola et al. 2007b). The experimental area was located on the northwest face of the valley at an elevation between 1,010 and 1,030 m a.s.l. with a steep slope (33°). Soils are xerochrepts with clay loam texture (49% sand, 32% silt, 19% clay) and relatively high gravel content of 46% (Barba et al. 2013; Sus et al. 2014). The substrate consists of fractured metamorphic schist that outcrops on a large part of the study area. Mineral soil is slightly acidic (pH is 6.2) and contain 5.9% of C, 0.27% of N and 0.044% of P (Curiel Yuste, unpublished results). No differences are found in C, N or P availability or in soil carbon pools (both quality and quantity) between soils associated with the different type of trees (Curiel Yuste et al. 2012). For more information related to the study area, see Hereter and Sánchez, 1999; Barba et al., 2013.

This mixed forest is mainly dominated by Scots pine (*Pinus sylvestris* L.) in the overstorey and holm oak (*Quercus ilex* L.) in the understorey, with total stem density of 2235 stems ha^{-1} (Poyatos et al. 2013). Severe drought events since the 1990s (Martínez-Vilalta and Piñol 2002) and particularly in 2001-2003 and 2005-2008 (Hereş et al. 2012) have affected the Scots pine populations, inducing a mean crown defoliation of 52% and standing mortality of 12% (Vilà-Cabrera et al. 2013). This situation, coupled to the low regeneration of pines (Vilà-Cabrera et al. 2013) is currently driving the replacement of pines by oaks as the dominant overstorey species.

2.2. Experimental design

2.2.1 Experiment scheme

165 Soil respiration fluxes were measured close to 12 trees (less than two meters from the
166 tree stem) belonging to the different stages along the vegetation shift following the
167 Scots pine die-off process (from now, type of tree): three non-defoliated Scots pines
168 [NDP], three living defoliated Scots pines [DFP], three dead Scots pines [DP] and three
169 Holm oaks [HO]. The maximum distance between measuring points was *ca*. 200 m.
170 Dead Scots pines were devoid of needles and small branchlets, and only the main bole
171 and primary branches were still standing. Hereş et al. 2012 found that 86% of the
172 standing mortality resulted from the drought events in 2001-2003 and 2005-2008.
173 Therefore we estimate that these trees have been dead for 3-11 years. The degree of pine
174 defoliation was visually estimated as the percentage of green needles relative to a non-
175 defoliated canopy of a similar sized tree from the same population (Galiano et al. 2010).
176 Defoliated pines had less than 50% of green leaves. DBH for each type of tree was 37 ± 7
177 cm in NDP, 59 ± 9 cm in DFP, 58 ± 7 cm in DP and 14 ± 8 cm in HO (mean $\pm$ sd). The
178 Hegyi competition index was significantly higher for HOs with respect to pines,
179 whereas no significant differences were found between NDP, DFP and DP (Curiel
180 Yuste et al. 2012).
181 The root excision method (Subke et al. 2006) was used for studying total, autotrophic
182 and heterotrophic soil respiration, using the protocol proposed by Heinemeyer et al.,
183 (2007). Three different PVC collars (treatment collars) of 63 cm in diameter were
184 installed within 3 m of each tree (Figure 1). The first type of collar (A) was 5 cm in
185 height and was inserted only 2 cm into the ground and fixed with three metal sticks.
186 Thus, these A-type collars did not interfere with fine roots, mycorrhizal or soil
187 microbial dynamics and growth. The second and the third types of collar (B and C
188 respectively) were 50 cm in height and were inserted up to a depth of 45 cm into the
189 soil. Collar B had four rectangular windows (17 x 5 cm) at 10 cm from the top, covering
190 33% of the total collar perimeter. Windows were covered with nylon fabric of 41 μm
191 mesh size, allowing ingrowth of fungal hyphae but not of roots (Ek 1997). The deeper
192 B-type collars prevented the ingrowth of fine roots (which are concentrated mainly in
193 the upper centimetres of the soil (Jackson et al. 1997). C-type collars were the same size
194 and were installed at the same depth as B-type collars, but they did not have windows,
195 so it prevented the ingrowth of both, fine roots and mycorrhizal hyphae. Hence, we
196 assumed that in C-type collars, only the non-rhizospheric microbial community
197 remained active.

198 Due to the great stoniness of the soil, two modifications were made to the Heinemeyer
199 et al., (2007) experimental design. First, treatment collars were bigger (20 cm in
200 diameter in the original protocol), and second, we dug a hole in the soil previous to the
201 installation of the deeper collars. After placing the collar inside the hole, we filled the
202 space inside the collar with the previously removed soil material, preserving the original
203 soil-horizon order. Two smaller PVC collars (sampling collars; 10 cm in diameter and 4
204 cm of height) were installed as replicate collars within each treatment collar. Sampling
205 collars were inserted 1 cm into the ground and fixed with polyurethane foam. These
206 collars delimited the reference surface of our soil respiration measurements. Collar
207 installation was made 14 months before the start of measurements in order to minimize
208 the effects of the soil disturbance on soil respiration measures.

209

210 2.2.2 Measurements
211 Soil respiration rates were measured with a close-path infra-red gas analyser (IRGA)
212 coupled to a closed dynamic chamber (EGM-4 and SRC-1, PP-Systems, HITCHING,
213 UK) in the sampling collars (the soil chamber fitted well with the small collars because
214 it had the same diameter). Soil respiration was measured every two weeks during one
215 year, from June 2012 to June 2013 (25 campaigns). For each campaign, five rounds of
216 measurements were made at all 72 small collars during 24 hours, in order to capture the
217 soil respiration variability associated to daily cycles. One round was started two hours
218 before sunrise, another was performed after sunset and the others were equally
219 distributed during the daytime. Since soil respiration was manually measured, the effort
220 for integrate the spatial variability of soil respiration in this extremely rocky location
221 and also the daily soil respiration course did not allow us to increase the number of
222 measured trees.
223 Soil temperature at 10 cm was measured once per big collar and per round of
224 measurements using a thermometer (OMEGA, HH806AU, Stamford, USA). Soil water
225 content (SWC) in the top 15cm was measured each campaign at each tree by time
226 domain reflectometry (TDR) (Tektronix 1502C, Beaverton, Oregon, USA). One 15 cm
227 long TDR probe was permanently installed vertically in the upper soil close to each tree
228 throughout the experiment. In order to correct the SWC measurements for the stoniness,
229 gravimetrical SWC measured in soil samples close to the TDR probes were regressed
230 against TDR measurements (for more information, see Poyatos et al., 2013).

231 One litterfall trap (555 x 355 mm) was installed within 2 m of each tree and litterfall

232 was collected during every campaign, dried during 24 h at 70°C and weighed.

233 Air temperature and relative humidity were continuously measured in a meteorological

234 tower installed less than 100 m from the farthest tree. Continuous SWC was also

235 recorded in the upper 30 cm of soil using four frequency domain reflectometers (CS616,

236 Campbell Scientific INC) randomly distributed among the trees. A data acquisition

237 system (CR1000 datalogger and AM16/32 multiplexers, Campbell Scientific Inc.,

238 Logan, UT, USA) was used to store 15-min means of soil moisture and meteorological

239 variables sampled every 30 s.

240

241 2.3. Data analysis

242 2.3.1. Soil respiration calculation

243 The five soil respiration measurements recorded for each small collar were time-

244 averaged in order to obtain mean daily soil respiration. Then, both replicates were

245 averaged at the big collar level (A, B and C; Figure 1).

246 Total soil respiration (R_S), heterotrophic soil respiration (R_H), autotrophic soil

247 respiration (R_A), fine roots respiration (R_R) and micorrhyzal respiration (R_M) were

248 calculated following the protocol proposed by Heinemeyer et al., (2007). R_S and R_H

249 were estimated directly as the soil respiration rates measured in collars A and C,

250 respectively. R_A was calculated by subtracting C from A; R_R by subtracting B from A

251 and R_M by subtracting C from B.

252

253 2.3.2. Soil respiration drivers.

254 Mixed-effects models were used to analyse the relationships between soil respiration

255 and type of tree, soil temperature, SWC, litterfall and season with campaign data. The

256 limits of seasons were adjusted from environmental variables (i.e. Summer ended with

257 the first rainfall events in Fall, which changed drastically SWC and temperature). Linear

258 and exponential relationships between soil respiration and temperature were tested in

259 the models, as well as linear and quadratic relationships between soil respiration and

260 SWC. In all cases, the linear relationships performed better than the nonlinear

261 transformations according to the AICc (corrected Akaike information criterion) statistic

262 (data not shown).

263 Different models were fitted for each soil respiration component (R_S, R_H, R_A, R_M and

264 R_R). As all variables were measured near the same trees throughout the campaigns, tree

265   identity was included as a random factor in all models. Models with all combinations of

266   predictor variables and their second-order interactions were performed, and the best

267   model in terms of AICc was selected. To determine the variability explained by each

268   mixed model, we calculated the coefficient of determination using the log likelihood of

269   both, the studied model and the null model (which did not include any predictor

270   variables). In the root exclusion method, the PVC collars could interfere with soil

271   temperature and moisture (Kuzyakov 2006). Unfortunately, due to instrumental

272   limitations, SWC was only measured at tree level (and not at collar level) at each

273   campaign. Although, SWC was gravimetrically measured at samples inside the 72 small

274   collars at the end of the experiment (July 2013) and one-way ANOVA was performed

275   to test for differences in SWC between the different treatment collars.

276

277   2.3.3. Annual SR

278   We used the models fitted in 2.3.2 to estimate daily values of R_S, R_H, R_A, R_M and R_R for

279   a whole year (from mid-June 2012 to mid-June 2013). Apart from fixed predictors

280   (season, type of tree), daily-aggregated values of soil temperature, SWC and litterfall

281   were needed as inputs for the model. For each tree and collar type, linear regressions

282   were fitted between daily-averaged soil temperature measured during the campaigns and

283   simultaneous air temperature measured at the meteorological tower (R^2=0.93±0.02,

284   across-trees mean±sd). Likewise, daily SWC for each tree was estimated from linear

285   regressions against mean SWC measured with the four frequency domain reflectometers

286   near the meteorological tower (R^2=0.66±0.27, across-trees mean±sd). Daily litterfall

287   across-trees was linearly interpolated from biweekly-measured litterfall. Modelled

288   values of daily R_S, R_H, R_A, R_M and R_R were then aggregated to obtain annual values.

289   Mixed-effects models, including tree identity as a random factor, were then used to

290   analyse the differences in annual soil respiration and its components between types of

291   tree. Given the high spatial variability of soil respiration, especially in this ecologically

292   complex site (Barba et al., 2013), and the limited number of replicates (3 trees per type),

293   we considered marginally significant differences among means when $0.05 < p < 0.1$.

294   Additionally, Friedman test and its post hoc analysis were applied to the daily-averaged

295   data from the 25 campaigns, to test for possible differences in soil respiration and its

296   components between types of tree. The Friedman test is a non-parametric repeated

297   measure ANOVA. Its procedure involves ranking soil respiration from the different tree

298   types and then considering the values of ranks by campaigns.

299   To test whether the relative contribution of R_H to R_S increases along the die-off process,

300   mixed-effects models with tree type (for testing annual differences) or with the

301   interaction between tree type and season (for testing differences over seasons) were

302   used, including tree identity as a random factor. Heterotrophic relative contribution data

303   was log transformed to achieve normality.

304   All the analyses were carried out using R 3.0.3. (R Foundation for Statistical

305   Computing, Vienna, Austria). The mixed-effects models were performed using the R

306   package nlme (Pinheiro et al. 2009) and the step-wise model selection was performed

307   with MuMIn package (Bartón 2014).

308

309   **3. Results**

310   3.1. Seasonal course of environmental variables and soil respiration components.

311   Over the study period, climate was typical of a low elevation Mediterranean mountain,

312   with mean air temperature of 11.2°C, annual precipitation of 703 mm and relatively dry

313   summer (93 mm from June to September and mean SWC below 15% from mid-July to

314   the end of September) (Figure 2b and 2c). The seasonal pattern of litterfall was not as

315   clear as those of temperature and SWC, but it seemed to peak at the end of fall (Figure

316   2d).

317   No significant differences were found for soil temperature, SWC and litterfall among

318   types of trees (Table 1). While type of collar did not influence soil temperature during

319   the experiment (p=0.87, repeated measures ANOVA), SWC measured gravimetrically

320   at the end of experiment (July 2013) was higher for deeper collars (B and C) than for

321   surface ones (A) (p=0.03, one-way ANOVA), 3.4% on average.

322   Total soil respiration (R_S) flux peaked during late spring and early summer (up to 6

323     $\mu mol\ m^{-2}\ s^{-1}$; Fig 3, top panel) and then decreased over the course of the summer to 33

324   % of peak values. During fall and winter R_S was quite low (between 0 to 2 $\mu mol\ C\ m^{-2}$

325   s^{-1}). R_H showed a similar annual pattern as R_S with values ranging between 1 and 4

326     $\mu mol\ C\ m^{-2}\ s^{-1}$ (Figure 3, R_H panel). No seasonal pattern was observed either for R_A, or

327   for its components (R_M and R_R) (Figure 3, bottom panels). Soil CO_2 efflux measured in

328   HO was the lowest in 88% of the campaigns for R_S, 76% for R_H, 68% for R_A, 56% for

329   R_R, but only in 24% of the campaigns for R_M (Figure 3). Indeed, the non-parametric

330   Friedman test applied to the campaign data indicated that respiration rates under HO

331   were lower than under the three types of pines for R_S, R_H, R_A and R_R (p<0.001 for R_S

332   and R_H, p=0.001 for R_A and p=0.002 for R_R), but not for R_M.

333   The relative contribution of R_H to R_S did not show differences among tree types, either

334   at annual (p=0.968) or at seasonal scales (p=0.325) (GLM models with tree identity as a

335   random factor). Nonetheless, the relative contribution of the different soil respiration

336   components considering all tree types together varied during the year. The contribution

337   of R_H to R_S was highest during late winter, spring and summer, and decreased during

338   fall and early winter. In contrast, the contribution of R_R to R_S increased during fall and

339   early winter. The contribution of R_M to R_S did not show any seasonal pattern, remaining

340   low throughout the year (Figure 4).

341

342   3.2. Soil respiration drivers.

343   A mixed-effects model including the interactions between season-SWC-temperature and

344   temperature-type of tree as predictor variables (Table 2, R_S) explained 51% of the

345   seasonal variability in R_S. The higher the SWC, the larger the positive effect of

346   temperature was on R_S, especially during spring and summer. However, at SWC values

347   below 10%, the temperature effect on R_S was negligible in summer (Figure 5; i, j, k and

348   l). Additionally, SWC had higher effect on R_S under HO than under pines. Similarly,

349   tree type interacted with SWC to determine R_H, while the rest of the R_H predictors were

350   almost the same as those in the R_S model (Table 2, R_H and Figure 6). R_H model

351   explained 56% of the total variability in R_H. The interaction between SWC and

352   temperature had the same positive effect than in the R_S model (higher effect of

353   temperature at high SWC values). Temperature had the lowest effect on R_H during fall

354   and winter, a higher effect during summer and the highest effect during spring (Table 2,

355   R_H).

356   Models of the autotrophic components of R_S explained much less variability than the

357   ones fitted for R_S and R_H. The selected R_A model was able to explain only 15% of the

358   autotrophic respiration variability, and included the positive effect of litterfall and the

359   positive interaction between SWC and temperature (Table 2, R_A). Likewise, the selected

360   R_R model was able to explain only 13% of the variability in fine root respiration and

361   contained the interactions season-SWC and season-temperature (Table 2, R_R). Finally,

362   the selected R_M model was able to explain 24% of the variability in mycorrhizal

363   respiration and contained only the interaction between SWC-temperature (Table 2, R_M).

364   Type of tree had no effect on R_A, nor on its fractions (R_R and R_M).

365

366   3.3. Annual soil respiration and its components.

367   For the overall set of sampled trees, modelled annual R_S (mean±SD) from July 2012 to

368   July 2013 was 2.6±0.6 µmol m^{-2}s^{-1}, R_H was 1.7±0.3 µmol m^{-2}s^{-1}, representing 65% of

369   R_S, while R_A was 1.0±0.5 µmol m^{-2}s^{-1} (36% of R_S). R_R and R_M, as components of R_A,

370   were 0.6±0.6 µmol m^{-2}s^{-1} (23% of R_S) and 0.3±0.7 µmol m^{-2}s^{-1} (13% of R_S),

371   respectively.

372   The mixed-effects models showed that annual R_S under holm oak trees (HO) was

373   marginally lower than under non-defoliated pines (NDP) (p-value: 0.074) (64% on

374   average), whereas defoliated pines (DFP) and dead pines (DP) did not show differences

375   with NDP or HO. Likewise, annual R_H under HO was significantly lower than under

376   NDP (p-value: 0.030; 36% lower), and marginally significantly lower than under DFP

377   (p-value: 0.089; 23% lower) and DP (p-value: 0.054; 33% lower). Mixed-effects models

378   for annual R_A, R_R and R_M did not show differences among types of trees (Figure 7).

379   We used the model in section 3.2 to assess the effect of the higher soil moisture in

380   deeper collars (B and C) due the collar effect (3.4% higher on average at July 2013),

381   because this increased SWC could have stimulated R_H, overestimating the heterotrophic

382   contribution to R_S and consequently, underestimating R_A. Since SWC was not measured

383   at the different collar types during the campaigns, it was not possible to asses this effect

384   over the year. However, if we assumed that the difference observed in July persisted

385   throughout the year, annual R_H and its contribution on R_S would only have been 0.92%

386   and 0.60% higher than the reported values, respectively (models estimations with an

387   increment of 3.4% in SWC for all campaigns). Therefore, we considered the collar

388   effect on the estimation of respiration fluxes to be negligible.

389

390   **4. Discussion**

391   4.1. Annual soil respiration and its fractions

392   Mean annual R_S at the study site was 2.6 μmols m^{-2} s^{-1}, similar to values reported for a

393   parallel valley in the same nature reserve (2.3 μmols m^{-2} s^{-1}, Asensio et al., 2007). R_H

394   was the most important fraction of R_S, representing about 65%. This relative importance

395   agrees well with estimates obtained in other forest ecosystems (Rey et al. 2002;

396   Tedeschi et al. 2006; Heinemeyer et al. 2007; Subke et al. 2011), confirming the

397   important role of microbial soil respiration in R_S. The R_R and R_M contribution to R_S

398   (23% and 13%, respectively) were also similar to values reported in other partitioning

399   studies (Malhi et al. 1999; Rey et al. 2002; Subke et al. 2006; Heinemeyer et al. 2007;

400   Ruehr and Buchmann 2010).

401

402   4.2. Soil respiration and its components following Scots pine die-off

403   Scots pine die-off, from non-defoliated pines to defoliated pines and to dead pines, was

404   not associated with major changes in soil respiration (both heterotrophic and autotrophic

405   activity, see Figure 7), contrary to what we had hypothesized (H1). Therefore, also the

406   relative contribution of heterotrophic respiration did not increase from NDP to DFP and

407   DP. Defoliation did not affect R_S or its components with respect to non-defoliated pines

408   (NDP). Moreover, 3-11 years after tree death, we observed that R_S (and all its

409   components) associated with dead Scots pines has either completely recovered or

410   remained unchanged (Figure 7). Even the autotrophic components (R_R and R_M) of R_S,

411   directly dependent on the substrate inputs from plant photosynthetic activity (e.g.

412   Högberg et al., 2001), were not affected along this die-off gradient. These effects are

413   surprising, given that soil autotrophic respiration had been reported to be extremely

414   sensitive to decreases in photosynthetic activity associated with defoliation and die-off

415   (Levy-Varon et al. 2012; Moore et al. 2013; Levy-Varon et al. 2014; Borkhuu et al.

416   2015). Indeed, decreases in plant productivity have been associated with lower fine root

417   biomass and hence lower root metabolic activity, lower belowground substrate

418   allocation and lower root exudation, all contribution to lower R_A and its fractions

419   (Högberg and Read 2006).

420   We did not find higher heterotrophic respiration (R_H) under DP than under NDP, which

421   is consistent with the fact that tree mortality did not apparently result in higher soil

422   moisture, soil temperature or litterfall under dead pines, factors which are known to

423   stimulate R_H. Although the relative contribution of R_H to R_S (and thus, also R_A
424   contribution) showed a clear seasonal pattern, this pattern was preserved across the
425   entire pine die-off gradient (NDP, DFP and DP).

426   The stability in R_S and in its fractions along the pine die-off gradient denotes a high
427   degree of resilience of soil processes with respect to aboveground perturbations. Some
428   studies have also shown that R_S remained stable after a perturbation (Binkley et al.
429   2006; Levy-Varon et al. 2014), suggesting that this R_S resilience could be produced by
430   mobilization of reserve carbohydrates (Levy-Varon et al. 2012), by a higher growth
431   rates of non-disturbed trees (Levy-Varon et al. 2014) speculating that an increment of
432   R_H due to an increment in organic matter availability could be compensating a decrease
433   in R_A (Borkhuu et al. 2015). While this has been partially shown in some studies (Levy-
434   Varon et al. 2012; Moore et al. 2013; Levy-Varon et al. 2014; Borkhuu et al. 2015),
435   here we present, to the best of our knowledge, the very first evidence that all the
436   components of R_S remain apparently unaffected after 10 yr of drought-related mortality
437   processes.

438   Our results might be partially explained by the disturbance recovery findings in Nave *et*
439   *al.* (2011), which suggest that a short perturbation of forest C cycling due to partial
440   canopy disturbance could be rapidly recovered (within a few years), thereby stabilizing
441   the C cycle. They hypothesize that shortly after the perturbation, the expected decrease
442   in ecosystem production and soil respiration (due to decrease fine root biomass and non-
443   structural carbohydrate concentrations in roots) could be compensated by higher growth
444   rates from the remaining healthy trees due to reduced competition for limiting
445   resources. At the study site, after drought-induced pine mortality in the 1990's higher
446   growth rates of the remaining healthy trees were observed (Martínez-Vilalta and Piñol
447   2002), consistent with this hypothesis.

448   The mechanism underlying the observed resilience of R_S following Scots pine die-off is
449   likely more related to belowground colonization by HO than to the remaining,
450   unaffected, pines. Results obtained from previous studies in the same site support this
451   idea. Firstly, spatial variability of R_S close to dead pines has been mainly associated
452   with the spatial distribution of HO basal area (Barba et al. 2013) suggesting a functional
453   colonization by HO rhizosphere. Moreover, it has been observed that rates of R_R
454   measured in living roots under DP were similar to those measured for HO (Pereira-
455   Blanco 2014), which suggests that fine roots colonization of the disturbed gap is taking
456   place by HO. Finally, Curiel Yuste et al. 2012 found that there was a similarity in the

457 most abundant bacterial taxon (i.e. Actynimycetes, Rhizobiales, Xantomonadales) between

458 rhizosphere from DP and HO, indicating that HO colonization is also occurring at the

459 microbial level.

460

461 4.3. Drought-induced substitution of Scots pine by holm oak causes a decrease in soil

462 respiration

463 Changes in vegetation could produce changes in the whole plant-to-soil system, such as

464 root biomass and distribution, nutrients and water balances, net primary production,

465 carbohydrate allocation patterns, litter quantity and quality, decomposer community or

466 microbial diversity (Jackson et al. 1997; Binkley and Giardina 1998; Palacio et al. 2007;

467 Strickland et al. 2009; Curiel Yuste et al. 2012), which in turn, could modify R_S and its

468 heterotrophic and autotrophic fractions (Uren 2000; Janssens et al. 2001; Kuzyakov

469 2006; Cornwell et al. 2008; Vivanco and Austin 2008). Despite the strong resilience of

470 R_S and its components along the Scots pine die-off (NDP, DFP and DP) (see section

471 4.2), changes in R_S associated with the succession from Scots pine to holm oak were

472 observed. Annual R_S was 36% lower in HO compared to NDP, contrary to our

473 hypothesis H2. Although the analysis of annual values did not show differences in R_A,

474 R_R or R_M between NDP and HO (Figure 7), seasonal data analysed with the non-

475 parametric Friedman test suggested lower values for R_A, and R_R under HO. However,

476 the magnitude of these differences was small (Figure 3) and the differences in R_S

477 between HO and NDP were apparently more related to differences in R_H (36% lower in

478 HO than in NDP). But the vegetation effect on R_S was not indirect via modifications in

479 the environmental conditions (as we expected in H3b), since no differences were found

480 in SWC or soil temperature along the die-off stages or between species. These

481 differences in R_S were probably driven by changes in microbial community composition

482 and functional diversity. This statement is supported by (i) the observed species-specific

483 microbial community under each type of tree at the study site and the lack of differences

484 in soil environmental conditions and in soil C pools (both quality and quantity) (Curiel

485 Yuste et al. 2012) and by (ii) the different functional diversity of the decomposer

486 community between type of trees as observed in a litter decomposition experiment in

487 the same study site (Barba et al. 2015).

488 This shift towards lower R_S under holm oak following Scots pine drought-induced

489 mortality could have crucial implications for the carbon balance of this particular

490 ecosystem and, by extension, for the carbon cycling in Mediterranean drought-exposed

Scots pine forests where a gradual replacement by *Quercus* species is increasingly being reported (Martínez-Vilalta et al. 2012; Galiano et al. 2013; Vilà-Cabrera et al. 2013; Carnicer et al. 2014). However, we aware that this study has been performed with limited number of replicates and studies addressing this question at ecosystem level should be required in order to make more reliable projections. Additionally, selecting individual trees as the experimental unit allows the comparison of soil respiration and its components between different types of trees, but hinders the extrapolation of the results to the forest level.

4.4. Environmental controls of autotrophic and heterotrophic soil respiration along a die-off gradient and species succession.

The interactions between season-SWC-temperature and temperature-type of tree were able to explain 51% of the R_S variability. The positive interaction between SWC and temperature indicated that the higher the SWC, the higher the temperature effect, and had similar effects on R_H, R_R and R_M fluxes. However, only during part of the spring-time were there simultaneous high SWC and temperature values (see figure 2). We did not find support for our hypothesized higher sensitivity of R_S and R_A to SWC under Scots pine (H2). On the contrary, we observe a higher sensitivity of R_H to SWC under HO (Table 2, R_H model; Figure 6), which could be due to a better adaptation to the strong seasonal changes in water availability of the microbial community associated with this typical Mediterranean species (Curiel Yuste et al. 2014) or to higher microbial biomass under HO resulting in a higher response to changes in SWC.

While environmental variables have been described as major drivers of R_A at global scale (Piao et al. 2010), vegetation activity has been usually described as the most determinant factor in R_S at ecosystem scale (Janssens et al. 2001; Tang et al. 2005; Högberg et al. 2009). Therefore, the limited explanatory power of environmental variables on autotrophic component models obtained in this study (and expected in H3a) could be explained by the lack of variables reflecting properly the photosynthetic activity of trees. Indeed a parallel study in the same area has shown that variations in R_R at tree level were positively correlated with the sap flow of the same trees (Pereira-Blanco 2014). This highlights the need for further studies on the dependency of R_S and its components on aboveground plant productivity (and not only on environmental variables), both measured at stand level, to understand the ecosystem mechanisms to cope with climate-driven disturbances.

536

537   **7. References**

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715

716 **Tables**

717 *Table 1*. Environmental variables during the study period summarized by type of tree

718 (mean and standard deviation). No differences has been found between type of trees

719 (p<0.05, mixed effects model with tree as random factor). NDP: non-defoliated pines;

720 DFP: defoliated pines; DP: dead pines; HO: holm oaks. Units: soil temperature in °C;

721 SWC in cm^3cm^{-3}; litterfall in $g\ m^{-2}\ d^{-1}$.

	NDP		DFP		DP		HO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temperature	11.68	0.29	11.70	0.68	12.00	0.40	11.21	0.14
SWC	20.20	0.04	20.21	0.06	20.21	0.02	20.20	0.05
Litterfall	1.84	0.67	1.19	0.11	1.92	0.89	1.96	0.19

722

Table 2. Summary of the selected model of R_S, R_H, R_A, R_R and R_M. NDP: non-defoliated pines; DFP: defoliated pines; DP: dead pines; HO: holm oaks. Holm oak and summer are used as the reference categories and are included in the intercept. Different lowercase letters indicate significant differences between levels of predictor variables.

R_S

	Variables	Estimate	SE	t-value	p-value	
	Intercept	6.971	2.016	3.458	0.001	
	SWC	-0.563	0.145	-3.888	<0.001	
	Temperature	-0.412	0.109	-3.798	<0.001	
Season	Summer	-	-	-	-	
	Fall	-8.275	3.410	-2.427	0.016	
	Winter	-5.869	2.640	-2.223	0.027	
	Spring	-4.132	3.392	-1.218	0.224	
Type	HO	-	-	-	-	
	NDP	1.102	0.669	1.647	0.138	
	DFP	0.426	0.673	0.633	0.545	
	DP	0.287	0.664	0.433	0.677	
	SWC*Temp	0.044	0.008	5.356	<0.001	
Season*SWC	Summer*SWC	-	-	-	-	
	Fall*SWC	0.596	0.182	3.269	0.001	
	Winter*SWC	0.536	0.162	3.305	0.001	
	Spring*SWC	0.423	0.196	2.161	0.032	
Season*Temp	Summer*Temp	-	-	-	-	
	Fall*Temp	0.840	0.339	2.480	0.014	
	Winter*Temp	0.476	0.328	1.450	0.148	
	Spring*Temp	0.347	0.270	1.285	0.200	
Temp*Type	Temp*HO	-	-	-	-	ab
	Temp*NDP	-0.024	0.027	-0.881	0.379	a
	Temp*DFP	0.016	0.027	0.608	0.544	ab
	Temp*DP	0.051	0.026	1.963	0.051	b
Season*SWC*Temp	Sum*SWC*Temp	-	-	-	-	a
	Fall*SWC*Temp	-0.050	0.015	-3.453	0.001	b
	Win*SWC*Temp	-0.043	0.015	-2.875	0.004	b
	Spr*SWC*Temp	-0.029	0.015	-1.949	0.052	ab

728

R_H

	Variables	Estimate	SE	t-value	p-value	
	Intercept	-0.734	0.578	-1.270	0.205	
	SWC	-0.032	0.029	-1.088	0.278	
	Temperature	0.027	0.033	0.826	0.410	
	SWC*Temp	0.009	0.002	1.146	0.000	
Season	Summer	-	-	-	-	
	Fall	1.344	0.617	2.179	0.030	
	Winter	1.455	0.574	2.536	0.012	
	Spring	-0.079	0.586	-0.133	0.894	
Type	HO	-	-	-	-	
	NDP	1.526	0.535	2.853	0.021	
	DFP	1.586	0.511	3.102	0.015	
	DP	1.667	0.479	3.480	0.008	
Season*Temp	Summer*Temp	-	-	-	-	[a]
	Fall*Temp	-0.112	0.051	-2.185	0.030	[b]
	Winter*Temp	-0.127	0.054	-2.345	0.020	[b]
	Spring*Temp	0.101	0.040	2.535	0.012	[c]
SWC*Type	SWC*HO	-	-	-	-	[a]
	SWC*NDP	-0.050	0.022	-2.289	0.023	[b]
	SWC*DFP	-0.061	0.021	-2.960	0.003	[b]
	SWC*DP	-0.059	0.019	-3.191	0.002	[b]

729

R_A

Variables	Estimate	SE	t-value	p-value
Intercept	1.611	0.586	2.747	0.006
Litterfall	0.076	0.035	2.204	0.028
SWC	-0.062	0.026	-2.379	0.018
Temperature	-0.095	0.037	-2.577	0.011
SWC*Temp	0.007	0.002	3.850	<0.001

730

731

R_R

	Variables	Estimate	SE	t-value	p-value	
	Intercept	-1.329	0.767	-1.731	0.0845	
	SWC	0.093	0.028	3.331	0.001	
	Temperature	0.041	0.028	1.463	0.145	
Season	Summer	-	-	-	-	[a]
	Fall	2.213	1.236	1.790	0.075	[ab]
	Winter	1.868	1.109	1.684	0.093	[ab]
	Spring	3.978	1.216	3.271	0.001	[b]
Season*SWC	Summer*SWC	-	-	-	-	[a]
	Fall*SWC	-0.123	0.053	-2.329	0.021	[b]
	Winter*SWC	-0.093	0.045	-2.077	0.039	[b]
	Spring*SWC	-0.128	0.046	-2.789	0.006	[b]
Season*Temp	Summer*Temp	-	-	-	-	[a]
	Fall*Temp	0.043	0.060	0.709	0.479	[ab]
	Winter*Temp	-0.070	0.063	-1.097	0.273	[ab]
	Spring*Temp	-0.182	0.045	-4.036	0.000	[b]

732

R_M

Variables	Estimate	SE	t-value	p-value
Intercept	1.286	0.544	2.366	0.019
SWC	-0.054	0.023	-2.395	0.017
Temperature	-0.081	0.032	-2.511	0.013
SWC*Temp	0.005	0.002	3.076	0.002

733

Figure captions

735 *Figure 1.* Root-exclusion experimental design. Large collars depict the different

736 exclusion treatments for soil CO_2 efflux measurements, small collars are

737 pseudoreplicates within treatments and small rectangles in B represent the mesh

738 allowing mycorhizae ingrowth. A, roots and mycorhizae included; B, roots excluded

739 and mycorhizae included; C, roots and mycorhizae excluded.

740

741 *Figure 2.* Seasonal course of environmental variables over the study period (June 2012

742 – June 2013). a) daily soil temperature (mean of all trees and treatments ± sd); b) daily

743 precipitation; c) daily SWC (mean of all trees ± sd); d) daily litterfall (mean of all trees

744 ± sd). Daily soil temperature for each tree and treatment was modelled with soil

745 temperature campaign data and daily air temperature. Similarly, daily SWC for each

746 tree was modelled with SWC campaign data and daily SWC measured continuously at 6

747 points randomly distributed in the study site (see section 2.2.2). Litterfall data showed at

748 panel d) are the campaigns data.

749

750 *Figure 3.* Annual soil respiration evolution and its components. a) Total soil respiration

751 [R_S]; b) Heterotrophic respiration [R_H]; c) Root respiration [R_R]; d) Micorrhyzal

752 respiration [R_M]. Each dot represents the average of the 5 measurements within a 24-h

753 cycle and the 3 trees of each type. Campaign-specific error bars were not drawn for

754 better clarity. NDP: non-defoliated pines; DFP: defoliated pines; DP: dead pines; HO:

755 holm oaks.

756

757   *Figure 4.* Soil heterotrophic respiration (R_H), micorrhyzal respiration (R_M) and root

758   respiration (R_R) contribution (in %) to total soil respiration (R_S) during campaigns (from

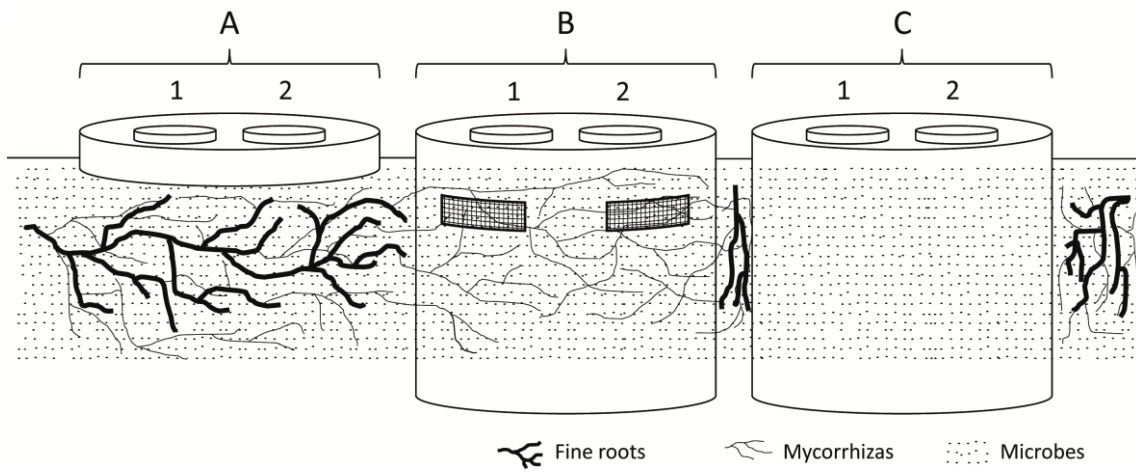759   June 2012 to June 2013) considering all tree types together.

760

761   *Figure 5.* Modelled soil temperature responses of R_S at different levels of soil moisture,

762   for each season and type of tree, according to the R_S model in Table 2. Modelled

763   responses have been drawn only for the observed soil temperature range for each

764   season. 10, 20 and 30% levels of SWC have been selected to cover the whole range of

765   SWC on the study site.

766

767   *Figure 6.* Modelled soil temperature responses of R_H at different levels of soil moisture,

768   for each season and type of tree, according to the R_H model in Table 2. Modelled

769   responses have been drawn only for the observed soil temperature range for each

770   season. NDP: non-defoliated pines; DFP: defoliated pines; DP: dead pines; HO: holm
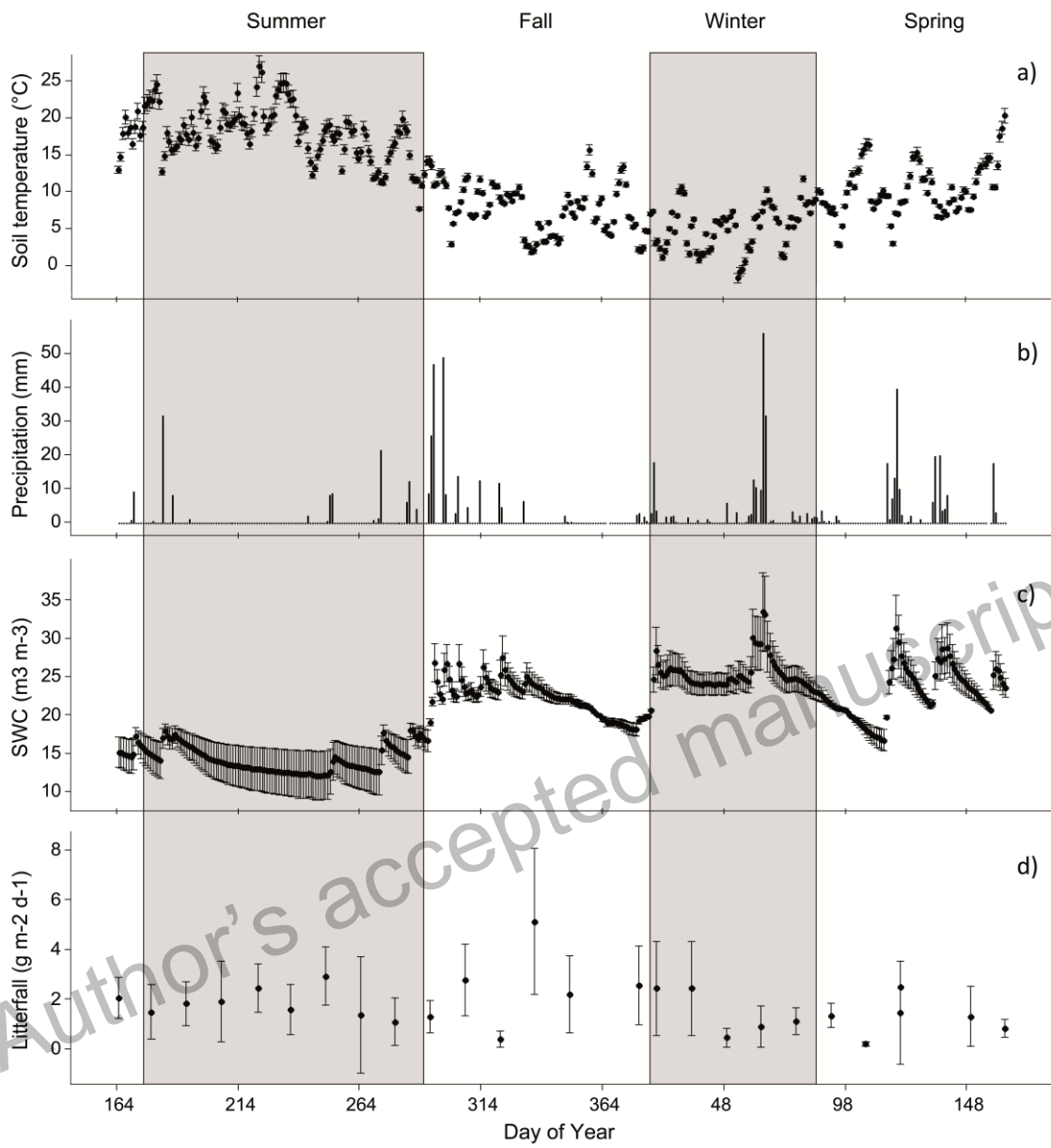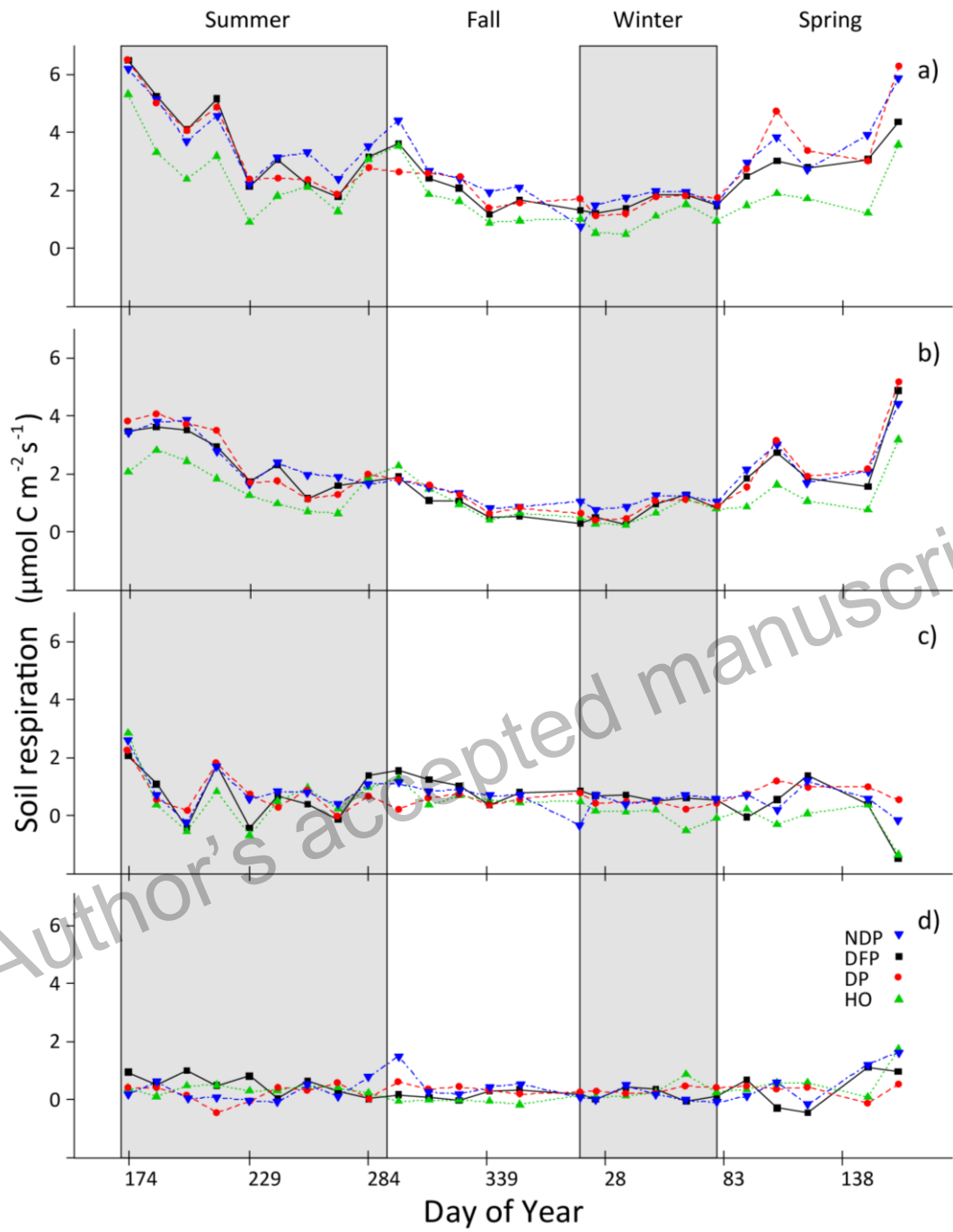
771   oaks.

772

773   *Figure 7.* Annual R_S and its components (mean ± sd) modelled for each type of tree.

774   NDP: non-defoliated pines; DFP: defoliated pines; DP: dead pines; HO: holm oaks.

775   Different normal font letters indicate significant differences between types of tree

776   within each respiration component ($p<0.05$, mixed-effects model). Different letters in

777   italics indicate marginally significant differences ($p<0.1$, mixed-effects model).

778
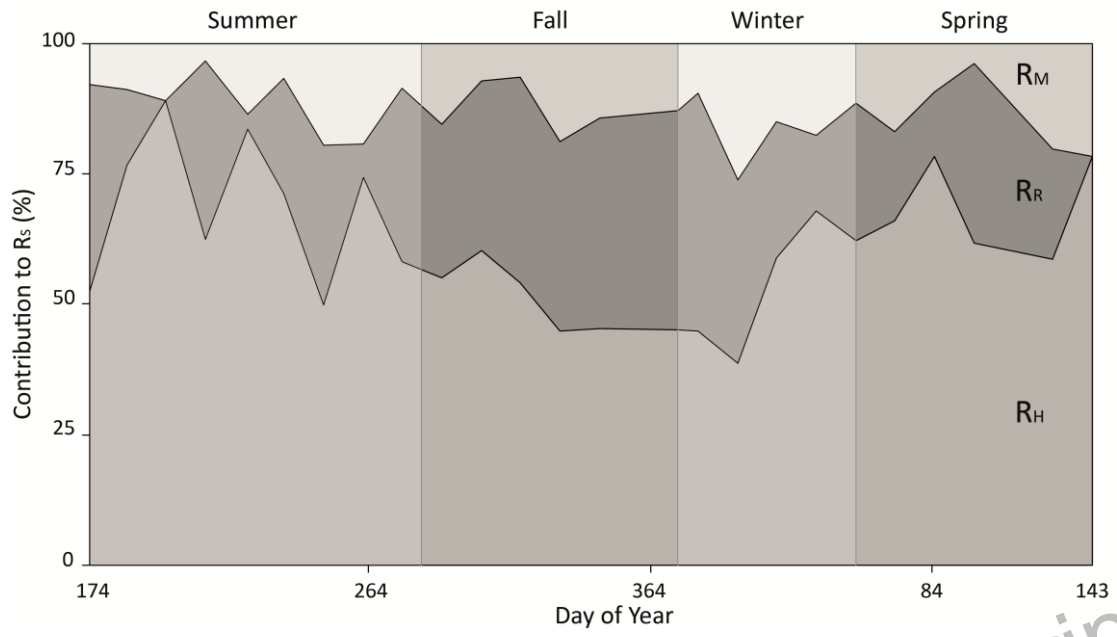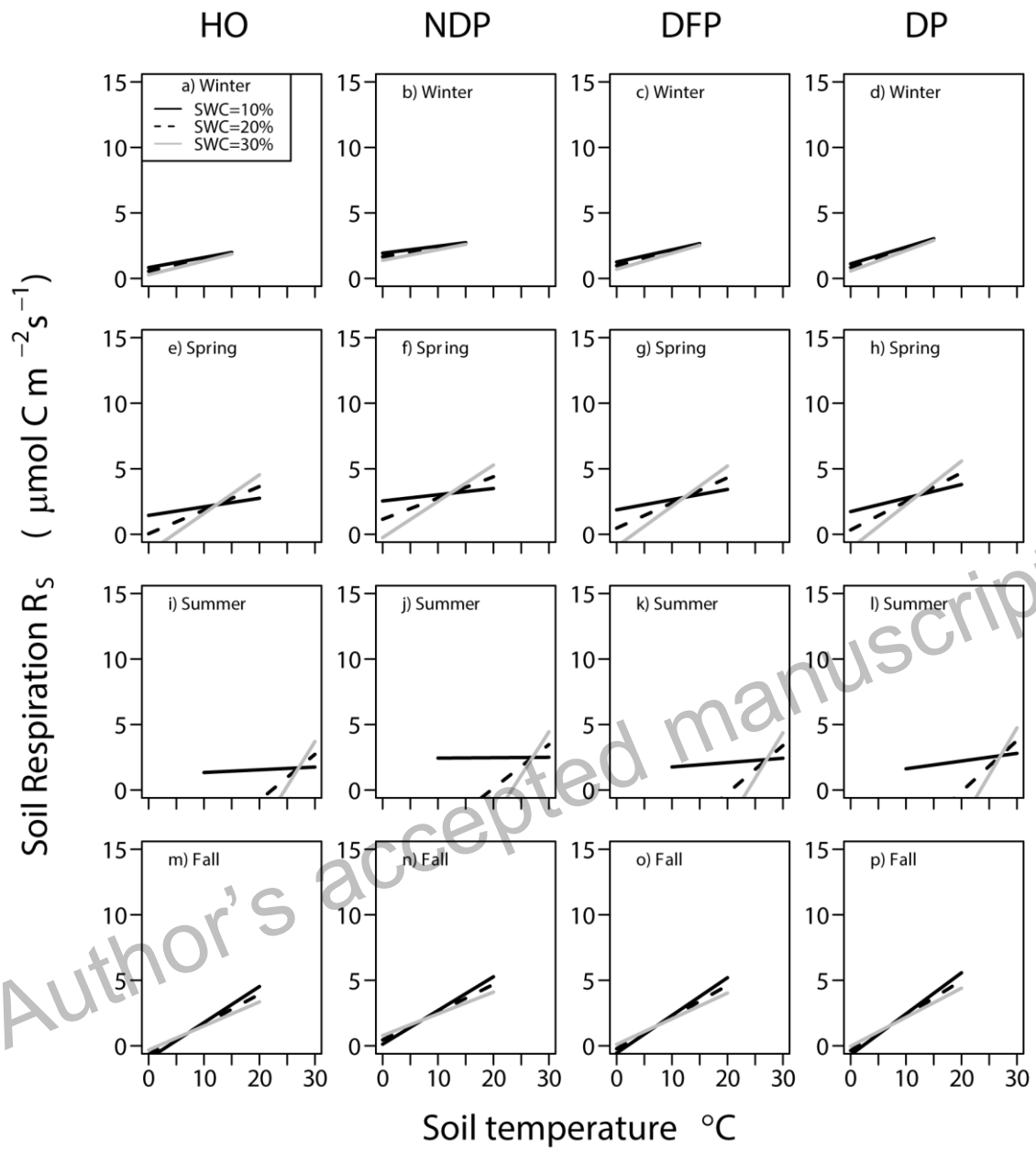
779   *Figure 1*

780

Figure 2

783   *Figure 3*

Figure 4

787　*Figure 5*

788

789

Figure 6

792 *Figure 7*

793