

1 **Effects of drought-induced forest die-off on litter decomposition.**

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9

10 **Summary**

11 *AIMS:* Drought-induced forest die-off and subsequent species replacement may modify
12 environmental conditions and eventually affect litter decomposition. We aimed to disentangle
13 the effects of tree species and die-off state on litter decomposition in a mixed forest where
14 *Pinus sylvestris* populations experiencing severe drought-induced die-off are being replaced by
15 *Quercus ilex*.

16 *METHODS:* Litter bags with leaves and fine roots from both species were placed under
17 canopies representing three habitats of the die-off and replacement process (healthy and dead
18 *P. sylvestris* and healthy *Q. ilex*). Mass was assessed over three years.

19 *RESULTS:* Species-specific chemistry of litter (C:N ratio) had a direct effect on mass loss, but
20 also indirect effects, attributed to the decomposer microbial community associated with a
21 given habitat-species. In their respective original habitats, oak leaves decomposed 44% faster
22 than pine needles, whereas oak roots decomposed 46% slower than pine roots.

23 *CONCLUSIONS:* Forest die-off and species replacement affected litter decomposition. This
24 effect can have great implications in forest functioning, particularly if drought-induced die-off
25 worsens in the next decades, according with the trend observed in the studied system.

26

27 **Key-words:** litter decomposition, forest die-off, home field advantage, carbon cycle,
28 Mediterranean forest.

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29 Introduction

30 The aerobic decomposition of dead organic matter is one of the main sources of CO₂ emission
31 from terrestrial ecosystems, consequently playing a critical role in their carbon (C) and nutrient
32 balances at both local (Santa Regina 2001; Bonanomi et al. 2010) and global scales (Prentice et
33 al. 2001; Canadell et al. 2007; Stocker et al. 2013). Therefore, in order to improve current
34 predictions of ecosystem responses to climate change, it is crucial to understand the drivers
35 controlling litter decomposition dynamics (Cao and Woodward 1998). Since the first half of the
36 twentieth century, temperature, moisture and vegetation have been described as the main
37 drivers of litter decomposition (Waksman and Gerretsen 1931). The key role of temperature
38 and moisture is based on the well-known fact that the enzyme kinetics involved in microbial
39 decomposition are very sensitive to both water and temperature (Davidson and Janssens
40 2006).

41 Furthermore, vegetation controls organic C decomposition in different ways. Firstly, vegetation
42 determines decomposition via species-specific litter quality, because the differences in
43 chemical litter composition between plant species imply different litter degradability, and
44 hence different rates of decomposition. This influence of litter quality on litter decomposition
45 has been described from local (Saura-Mas et al. 2012; Wang et al. 2014) to regional scales
46 (Melillo et al. 1982; Vivanco and Austin 2006; Cornwell et al. 2008). Accordingly, different
47 indicators of litter quality such as C:N ratio, nutrient (N and/or P) content, and the content of
48 some structural molecules (e.g. lignin or holocellulose), have been correlated with litter
49 decomposability (Gallardo and Merino 1993; Couteaux et al. 1995; Gholz et al. 2000; Vivanco
50 and Austin 2006; Bonanomi et al. 2010)). More specifically, litter's initial C:N ratio has been
51 identified as one of the best chemical predictors of litter decomposition (Melillo et al. 1982;
52 Parton et al. 2007; Berg and McClaugherty 2008; Bonanomi et al. 2010). Moreover, for a given
53 species, different organs (i.e. leaves, fine roots and twigs) present a different chemical
54 composition and, therefore, different rates of decomposition (Vivanco and Austin 2006;

55 Freschet et al. 2013; Wang et al. 2014). Secondly, vegetation has the ability to modify
56 environmental conditions, such as temperature and moisture (Binkley and Giardina 1998; Yuan
57 et al. 2012), thus indirectly determining decomposition by affecting enzyme kinetics (Cornwell
58 et al. 2008; Freschet et al. 2012) or photodegradation rates associated with exposure to
59 radiation (Austin and Vivanco 2006). Thirdly, vegetation can influence litter decomposition via
60 its co-evolution with the soil decomposer community (Vivanco and Austin 2008; Ayres et al.
61 2009a), resulting in specific tree-species soil communities (Grayston and Prescott 2005;
62 Waldrop and Firestone 2006; Curiel Yuste et al. 2012) with different functional diversity
63 (Waldrop and Zak 2004; Wallenstein et al. 2013). This co-evolution between tree species and
64 their soil communities is reflected by the capacity of specific microbial community to
65 decompose more efficiently the litter of the plant species from which is derived (Austin et al.
66 2014). This effect, called home-field-advantage (HFA) (Ayres et al. 2009a; Ayres et al. 2009b;
67 Austin et al. 2014), is widespread in forest ecosystems, enhancing litter decomposition by 8%
68 on average (Ayres et al. 2009b). However, how soil communities are able to efficiently
69 decompose different substrates and how differences in litter degradability could influence the
70 correct interpretation of HFA is still under debate (Freschet et al. 2012; Keiser et al. 2014).

71 All these factors highlight the complexity of the controls of litter decomposition dynamics,
72 which drives the C sink capacity of soils from terrestrial ecosystems, and the paucity of our
73 knowledge of above-belowground interactions. Another major source of uncertainty, for
74 instance, is that most research has focused on the decomposition patterns of above-ground
75 litter (needles and/or leaves), whereas the decomposition of fine roots, which accounts for at
76 least half of the litter produced by vegetation (Montero et al. 2005; Clemmensen et al. 2013),
77 and for most of the C incorporated in soil in the long-term (Clemmensen et al. 2013) has been
78 only marginally studied. In this regard, it is important to understand how climate change-
79 induced shifts in vegetation health (Lloret et al. 2012) may alter above-belowground
80 interactions and hence rates of organic matter decomposition and nutrient turnover. Drought-

81 and heat-induced tree die-off and mortality have been reported over the last few decades
82 around the world (Allen *et al.* 2010), particularly in South Europe and the Mediterranean Basin
83 (Lloret *et al.* 2004; Della-Marta *et al.* 2007; Briffa *et al.* 2009; Carnicer *et al.* 2011). Changes
84 towards ecosystems with a more limited supply of water (Giorgi and Lionello 2008; Mariotti
85 2010) have been associated with the decline of keystone species that have their southern limit
86 of distribution in the Mediterranean Basin (Lenoir *et al.* 2010; Vayreda *et al.* 2013; Carnicer *et al.*
87 *et al.* 2014). This is the case with *Pinus sylvestris* L., which, in some areas of the Iberian peninsula,
88 is being replaced by other species such as *Quercus ilex* L., which are better adapted to drought
89 (Vilà-Cabrera *et al.* 2013; Carnicer *et al.* 2014). Whereas many studies have speculated about
90 the possible significant impact on C and nutrients dynamics in vegetation shifts induced by
91 climate change (Cornwell *et al.* 2008; Ayres *et al.* 2009a; Ball *et al.* 2009; Ayres *et al.* 2009b;
92 McLaren and Turkington 2010; Freschet *et al.* 2013), really few studies have been directly
93 designed to estimate how climate-change-induced secondary succession may affect forest C
94 dynamics and the capacity of terrestrial ecosystems to sequester C (Díaz-Pinés *et al.* 2014).
95 The aim of this study is to assess how changes in litter quality associated with climate-change-
96 induced vegetation shifts affect litter decomposition rates. We addressed the climate-driven
97 forest succession from *P. sylvestris* to *Q. ilex* occurring in the Prades Mountains (NE Iberian
98 Peninsula). This information on litter decomposition in *P. sylvestris* forests is particularly
99 relevant for regional assessment of the C and nutrient balance because this widely distributed
100 species is experiencing severe die-off episodes in different regions (Martínez-Vilalta and Piñol
101 2002; Bigler *et al.* 2006). Leaf (senescent) and fine-root (fresh) litter bags from both species
102 were placed beneath healthy *P. sylvestris*, dead *P. sylvestris* and *Q. ilex* canopies in a fully
103 crossed factorial design, and decomposition and C and N content over three years were
104 measured. Specifically, we tested if drought-induced forest succession modifies litter
105 decomposition through changes in litter quality (litter effects), changes in the soil environment
106 (habitat effects) and their interaction.

107 **Materials and methods**

108 Our experiment was performed in a mixed forest on the northwest-facing hillside in Titllar
109 Valley, Prades Mountains (NE Iberian Peninsula; 41°13'N, 0°55'E; 1015 m asl). The climate is
110 Mediterranean, with a mean annual temperature of 11.3°C and mean annual precipitation of
111 664 mm (period 1951-2010) (Ninyerola et al. 2007a; Ninyerola et al. 2007b). The experimental
112 area was located on a 35° hill slope, on metamorphic schist substrate that outcrops onto a
113 large part of the study site. Soils are xerochrepts with clay loam texture and high gravel
114 content (46% volume). Organic horizons cover most of the soil and outcrops with variable
115 thickness. For more information about the studied area, see Hereter & Sánchez (1999) and
116 Barba *et al.* (2013). The mixed forest, which has not been managed for the last 30 years (Heres
117 et al. 2012), is mainly composed of *Pinus sylvestris* L. (Scots pine) (54% of the forest basal area
118 and mean diameter at breast height [DBH] of 0.32 m) and *Quercus ilex* L. ssp *ilex* (Holm oak)
119 (41% of the total BA and DBH of 0.15 m). The study area has been affected by several drought
120 events since the 1990s, particularly the *P. sylvestris* population (Martínez-Vilalta and Piñol
121 2002), producing an average mortality of 12% of standing trees and mean crown defoliation of
122 52% (Vilà-Cabrera et al. 2013). This situation, coupled with contrasted recruitment rates
123 between both species (low rates in *P. sylvestris* and high rates in *Q. ilex*) (Vilà-Cabrera et al.
124 2013), is leading to a progressive replacement of *P. sylvestris* by *Q. ilex* as the dominant over-
125 storey species.

126 The decomposition experiment considered three types of trees, representing different stages
127 of the ongoing forest succession (Healthy *P. sylvestris* [HPs], Dead *P. sylvestris* [DPs] and *Q. ilex*
128 [Qi]). We established two meters around trees as the respective rhizosphere-influence area on
129 soil environment (hereafter, habitat). Five replicates (hereafter, microsities) of each of these
130 three habitat types were selected on a 1-ha study site. Microsities of the three habitat types
131 were spatially randomized since die-off pattern was diffused. Selected dead pines had died in
132 the nineties as consequence of severe drought events (Martínez-Vilalta and Piñol 2002).

133 To assess litter mass loss over time we use the litter bags method. Despite the suitability of
134 this method for separating the effects of litter decomposition from microbially-stabilized plant-
135 derived tissue or losses of fragmented tissues through the mesh pores is under debate (Cotrufo
136 et al. 2013), is the most used method to study litter decomposition (Cotrufo et al. 2009),
137 specially indicated for field experiments allowing a large number of replicates.

138 Freshly senescent needles, leaves and living fine roots (diameter thinner than 2 mm) from *P.*
139 *sylvestris* and *Q. ilex* were collected from the same study area and oven-dried at 60°C for 24h.
140 Litter bags (0.5 mm nylon mesh and size 7.5 cm X 8.5 cm) were filled with a known dry-weight
141 amount of litter (0.5-1 g) (*Ps* needles, *Qi* leaves, *Ps* roots and *Qi* roots). Mesh size was large
142 enough to allow microbial and fungi activity as well as small access by arthropods, but small
143 enough to avoid major losses of the smallest litter portions (Killham 1994). Six litter bags
144 containing each litter type were placed on each microsite, with a total of 360 litter bags (3
145 habitat types X 5 microsite replicates X 4 litter types X 6 litter-type replicates). A square metal
146 fence (1 m X 1 m) was installed on each microsite and litter bags were placed inside to avoid
147 disturbances from wild boars during the experiment. Leaf litter bags were placed on the
148 surface and fine-root litter bags were buried at a depth of 5-10 cm. We did not remove either
149 the organic horizons underneath the litter bags at the beginning of the experiment or the
150 litterfall during the experiment - as commonly done (i.e. Vivanco & Austin 2008) - since we
151 wanted to mimic the natural conditions of the decomposition process as accurate as possible.
152 Litter bags were placed in the field in July 2011 and collected 0.16, 0.5, 1, 1.5, 2 and 3 yrs after
153 bags bury and oven-dried at 60°C for 24h. The remaining litter was dry cleaned with a brush
154 and weighed. Each individual sample was ground and analysed for total carbon [%C] content
155 by CHNS organic microanalysis, using combustion coupled with gas chromatography
156 (EUROVECTOR, EA3011, Milano, Italy). Similarly, initial litter quality (%N and %C) was assessed
157 for three samples of each litter type.

158 To control the possible effects of the microsites' environmental differences on decomposition
159 process, soil water content (SWC) and soil temperature were measured every two weeks from
160 January 2012 to July 2013 at each microsite. SWC was measured by time domain reflectometry
161 (TDR) (Tektronix 1502C, Beaverton, Oregon, USA). One TDR probe 15cm long was permanently
162 installed in the upper soil on each microsite. In order to correct the SWC measurements for
163 stone content, gravimetric SWC was regressed against TDR measurements (for more
164 information, see Poyatos et al (2013). Soil temperature was measured at 10 cm, using a
165 thermometer (OMEGA, HH806AU, Stamford, USA). Additional information about soil
166 properties such as pH, N availability, SOM content, soil bacterial community composition at
167 the different habitats could be found in Curiel Yuste et al (2012).

168 To assess the mass loss for litter and habitat types, we used a general linear model (GLM)
169 coupled with an exponential decay equation (expressed as $\ln(M_t / M_0)$, where M_t is the
170 remaining dry mass of each sample on the sampled date and M_0 is the initial dry mass) for
171 each litter type (fixed factor) and habitat type (fixed factor) (Saura-Mas et al. 2012), and we
172 included time as an additional variable in the model. Since the $\ln(M_t / M_0)$ divided by time has
173 been defined as the decomposition constant (k) (Olson 1963), the modelled slope for each pair
174 of combinations (4 litter types X 3 habitat types) represents the decomposition constant k of
175 each combination. As all the litterbags were collected on the same microsites throughout the
176 experiment, microsite was also included in the model as a random factor. The model also
177 contained the interactions between litter type, habitat type and time. Since this interaction
178 was significant, the effect of litter was analysed separately in an additional model that included
179 microsite and habitat type as random factors and time. Similarly, a model for habitat type was
180 built, including microsite and litter type as random factors and time.

181 Moreover, the remaining mass (%) was analysed by GLM models. The temporal pattern of
182 remaining mass was analysed separately for litter and habitat types in different models, which
183 also included microsite as a random factor and time. These models also tested differences

184 between litter and habitat types, respectively, for each collection event. Overall differences
185 between litter or habitat types for the whole period of time were analysed with similar GLM
186 models, but considering time as a random factor. A log-odd transformation was applied to
187 achieve normal distribution in the % of remaining mass (i.e. $\log[x/(1-x)]$).

188 To test the possible effect of initial litter quality on the decomposition process, linear
189 regression was fitted with the mean (\pm SE) of the k values obtained in each habitat type (n=3)
190 and mean (\pm SE) C:N values obtained in three samples analysed at the start of the experiment.

191 All the analyses were carried out using R 3.0.3. (R Foundation for Statistical Computing, Vienna,
192 Austria). The mixed-effects models were performed using the R packages nlme and lme4
193 (Pinheiro et al. 2009; Bates et al. 2014).

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194 **Results**

195 *Litter decomposition rates*

196 The decomposition rate of the different litter types varied between habitats, as supported by a
197 significant interaction between litter and habitat effects (Table 1, adjusted R^2 of the model was
198 0.75). All litter types were decomposed with a similar k in HPs habitat, but there were
199 differences in the DPs and Qi habitats (Fig. 1). In both the DPs and Qi habitats, Qi leaves and Ps
200 roots showed consistently higher decomposition rates than Ps needles and Qi roots. In the Qi
201 habitat, Qi leaves showed the highest decomposition rate, followed by Ps roots, and Qi roots
202 showed the lowest decomposition rates, whereas Ps needles showed intermediate rates
203 between the Ps roots and Qi roots. The Ps needle k was higher in HPs than in DPs habitats and
204 showed intermediate values in the Qi habitat. Similarly, Qi leaves were decomposed faster in
205 Qi habitats than in Ps habitats. The k of Ps roots did not show any significant differences in any
206 of the habitats, and Qi roots showed higher k in both the Ps habitats (HPs and DPs) than in the
207 Qi habitat. Overall, decomposition rates varied across the different litter origins: in Ps litter,
208 the decomposition rates were higher in needles ($0.14 \pm 0.01 \text{ yr}^{-1}$) than in roots ($0.17 \pm 0.01 \text{ yr}^{-1}$),
209 while the opposite trend was observed for Qi leaves ($0.19 \pm 0.02 \text{ yr}^{-1}$) and root ($0.13 \pm 0.01 \text{ yr}^{-1}$)
210 (GLM with litter type as predictor and microsite and habitat type as random factors and time,
211 $p < 0.05$) (Fig. 2a). Furthermore, when considering all three habitats together, litter composition
212 (k) almost significantly correlated ($R^2 = 0.76$; $p = 0.081$) with the initial litter quality (C:N ratio)
213 (Fig. 3). When the four litter types were considered together, no differences appeared in
214 decomposition rates between habitat types (Fig. 2b) (GLM with habitat type as predictor and
215 microsite and litter type as random factors, $p > 0.05$). No differences were found in the
216 measured environmental conditions between habitat types (GLM with soil temperature or soil
217 water content as independent variables, habitat type as predictor and microsite as random
218 factor; $p = 0.94$ for soil temperature and $p = 0.16$ for soil water content). Additionally, seasonal

219 patterns of soil temperature and SWC did not show differences among the different habitats
220 (Supplementary Material, Figure 1).

221 Since HPs and Qi represented the two forest successional extremes, the Ps needles and roots
222 decomposition rates beneath HPs were compared with the Qi leaves and roots decomposition
223 rates beneath Qi. Ps needles in the HPs habitat showed on average of 44% lower k than Qi
224 leaves in the Qi habitat ($p=0.046$). Ps roots in the HPs habitat showed on average 46% higher k
225 than Qi roots in the Qi habitat ($p=0.046$).

226

227 *Mass remaining over time*

228 The mass remaining over time decreased for all litter types (Fig. 4a) and habitats (Fig. 4b). Its
229 temporal evolution varied between litter types ($F=16.58$, $p<0.001$) but no significant
230 differences were found between habitat types for the whole time period ($F=1.56$, $p=0.250$,
231 GLMs with litter types and habitat types as predictors and microsite and time as random
232 factors). Qi roots maintained the highest remaining mass throughout the studied period, while
233 Qi leaves presented an accelerated biomass loss in comparison to the other litter types around
234 1.5 years after starting. The biomass loss of the two types of Ps litter remained quite similar
235 over the three years, with a tendency towards an increase in roots after 1.5 years.

236

237 **Discussion**

238 Here, we show that drought-induced secondary succession from *P. sylvestris* to *Q. ilex* may
239 substantially alter patterns of litter decomposition and N dynamics in Mediterranean forests.

240 This alteration may be due to both differences in litter quality between these two tree species
241 and the differential capacities of the microbial communities associated with the habitats to
242 decompose the different litter types.

243 Litter quality exerted a major control over litter decomposition, which is something that has
244 already been widely observed both at local (Gallardo and Merino 1993; Bonanomi et al. 2010;

245 Aponte et al. 2012) and regional scales (Couteaux et al. 1995; Vivanco and Austin 2006;

246 Cornwell et al. 2008). In our study, initial litter C:N ratio appeared as a good predictor of litter

247 quality since it correlated quite well with the decomposition rate constant (k) of the litter types,

248 independently of habitat. However, other chemical controls on litter decomposition may also

249 be important - for instance, initial lignin content, which is usually negatively correlated with

250 decomposition constant (Cornwell et al. 2008). In fact, lignin content in Ps needles has been

251 reported to be higher than in Qi leaves (Kattge et al. 2011; Mediavilla et al. 2011), in

252 agreement with our observations of the lower decomposition rates of Ps needles. Other

253 physical controls could also underlie the differences in litter decomposition observed

254 (Cornwell et al. 2008). Qi leaves show higher area/volume ratio than Ps needles (Kattge et al.

255 2011), enhancing microbial accessibility and consequently decomposition rates. Leaf litter

256 usually decomposes better than fine-root litter (Gholz et al. 2000; Vivanco and Austin 2006;

257 Freschet et al. 2013) due to its better quality (i.e. low C:N) (Bird and Torn 2006; Wang et al.

258 2010), as we observed in *Q. ilex*, but not for *P. sylvestris*, although the latter's needles showed

259 higher C:N than fine roots.

260 The lack of any significant differences in the abiotic environmental variables between habitats

261 points to the role of habitat specificities of soil decomposer communities as major controllers

262 of the observed differences between habitats in decomposition rates (Curiel Yuste et al. 2012;

263 Keiser et al. 2014). This statement is partially supported by the habitat-specific soil bacterial
264 community found at the same study site (Curiel Yuste et al. 2012). It has been hypothesized
265 elsewhere that soil decomposer communities associated with the distinct stages of forest
266 succession exhibit a specific capacity to decompose litter of varying quality (Freschet et al.
267 2012; Keiser et al. 2014). In our case, we observed that soil decomposer communities under
268 HPs habitats were able to decompose litter of differing quality to similar rates, whereas soil
269 decomposer communities under DPs and Qi were more selective and significantly capable of
270 decomposing better litter of higher quality. Thus, these functional differences along the
271 drought-induced successional gradient endorse the existence of a concomitant microbial
272 succession in such habitats, as reported for soil bacteria communities in the studied forest
273 (Curiel Yuste et al. 2012) and in other ecosystems (Waldrop and Firestone 2006; Wickings et al.
274 2012; Keiser et al. 2014). Despite information of fungal diversity and composition associated at
275 the different stages of pines die-off and Holm oak replacement at the study site is not available,
276 soil fungal communities are usually even more tree-species specific than bacterial communities
277 (Urbanová et al. 2015), reinforcing the idea that every habitat has a specific soil microbial
278 community (both bacterial and fungal).

279 Therefore, litter decomposition rates in forest subjected to drought-induced species
280 replacement would be modified not only by alterations in litter quantity and quality, but also
281 by changes in the decomposer community associated with tree replacement.

282 However, despite the differences in litter decomposition between microbial communities
283 seem quite clear, some uncertainties on the bacterial vs. fungal relative contribution remained
284 still unsolved, especially in a drying context. While soils of Scots pines forests have been found
285 more dominated by fungus than by bacteria compared to *Quercus* forests in a similar forest
286 succession type (Díaz-Pinés et al. 2014), fungal communities have been reported to be more
287 drought-resistant than bacterial communities (Wilkinson et al. 2002; Curiel Yuste et al. 2011;
288 Flores-Rentería et al. 2015; Tardy et al. 2015) and thus, fungal relative proportion increases in

289 drier conditions (Jensen et al. 2003). How these changes in bacterial-fungal communities due
290 to drought-induced shifts may affect ecosystem functioning such as litter decomposition is still
291 unclear and deserves further studies.

292 Our results only partially supported the HFA hypothesis, which proposes higher litter mass loss
293 under the species producing a given litter type (at *home*) than under other species (*away*). Qi
294 leaves were more efficiently decomposed under "home" (Qi) than under "away" habitats (HPs
295 or DPs ; 36% difference, on average). Soil decomposer community under Qi seems, therefore,
296 more specialized in the decomposition of higher quality organic matter (lower C:N ratio), such
297 as Qi leaves. However, the decomposer community in the HPs habitat seemed to be generalist,
298 and thus able to decompose litter of very different quality at similar rates (Figure 1) refuting a
299 potential local adaptation of the decomposer communities, as suggested by HFA hypothesis. .

300 Moreover, Ps roots showed similar decomposition rates across habitats, and for Qi roots, the
301 decomposition rates were actually higher in HPs than in Qi habitats. Overall, these results do
302 not support the HFA hypothesis, whereby the HFA should be, according to theory, more
303 pronounced in more recalcitrant litter (Strickland et al. 2009; Milcu and Manning 2011;
304 Strickland et al. 2013). Our results do concur, however, with 25% of the experiments that
305 performed reciprocal litter transplants between tree species without observing any
306 stimulation of decomposition at home (Ayres et al. 2009b).

307 The comparison of litter decomposition between the two forest succession extremes can help
308 to predict future trends in the C dynamics of these forests. While Qi leaves decompose faster
309 than Ps needles beneath their respective species, for fine roots the opposite trend was
310 observed: rates of fine-root decomposition in the HPs habitat were higher than the rates of Qi
311 fine-root decomposition in the Qi habitat, suggesting that secondary succession may produce a
312 substantial decoupling of above- and belowground trends in organic matter decomposition.
313 Thus, *P. sylvestris* replacement by *Q. ilex* would imply a faster decomposition of superficial
314 leaf-derived soil layers but a slower decomposition of root-derived material, which is generally

315 the major contributor to soil organic matter (Clemmensen et al. 2013). Nevertheless, the
316 amount of litter produced above and belowground by the different species was not measured
317 in this study, and the final C balance would ultimately depend on both, the decomposition
318 rates and the contribution of the above- and belowground biomass of the different species to
319 the C pool (Berg 2000; Bardgett et al. 2013).

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321 **Conclusions**

322 In this study, we are the first, to our knowledge, to use a real-case scenario of the effects of
323 die-off-driven forest succession on litter decomposition and N dynamics. Our study shows that
324 besides changes in soil temperature and moisture availability, climate change-driven
325 succession from *P. sylvestris* to *Q. ilex* is responsible for modifying both leaf and fine-root
326 decomposition through changes in the chemical nature of the litter and the relationship
327 between above-ground vegetation species and the belowground environment, including local
328 decomposer communities. In this particular study, drought-induced replacement of Scots pines
329 by Holm oaks seems to provoke significant changes, firstly in the chemical composition of litter
330 and secondly in the ability of different microbial communities to decompose organic matter.
331 The result should be a net increase in the decomposition rates of the "above-ground" litter
332 (moving from recalcitrant Ps needles to more palatable Qi leaves) but a net reduction in the
333 rates of "belowground" litter decay, largely due to the lower capacity of the microbial
334 communities under colonizer Holm-oaks to decompose root material as compared with those
335 under pines. Our results also suggest, therefore, that in order to correctly predict the effects of
336 climate change effects on C dynamics on forests, models should closely examine changes in
337 both the chemical composition and functioning of the decomposer communities associated
338 with drought-induced secondary successions.

339

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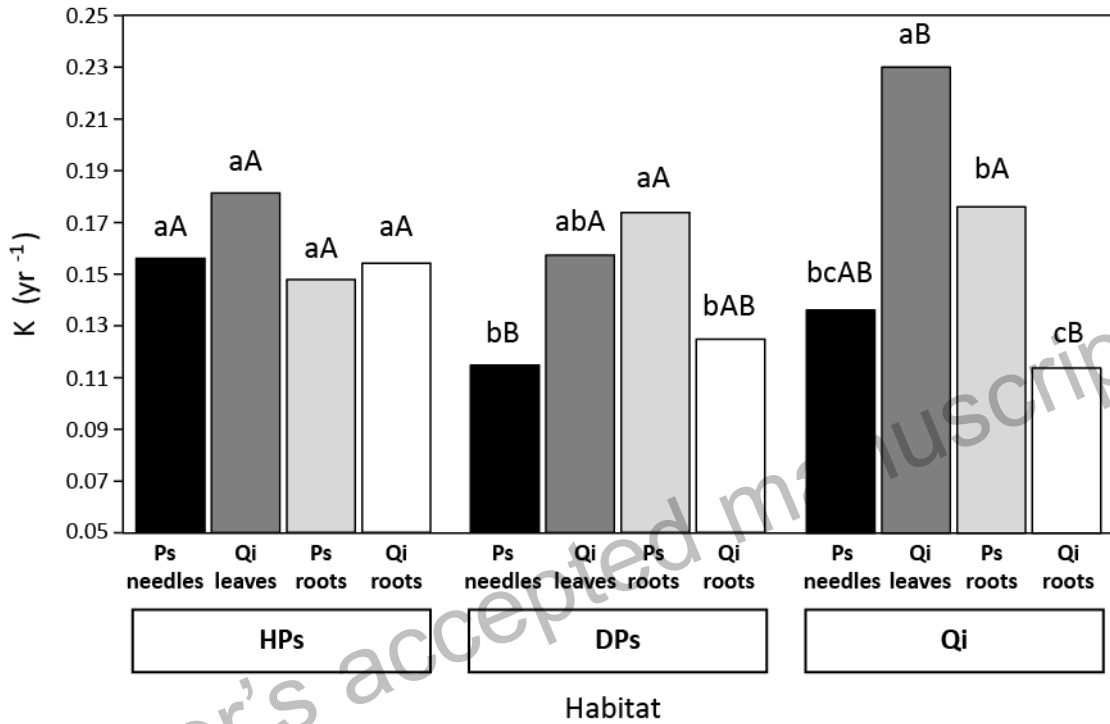
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558 **Figure legends**

559 **Fig. 1.** Decomposition constants (k) of *Pinus sylvestris* and *Quercus ilex* litter (leaves and fine
 560 roots) across three habitats obtained by the GLM. The lower case indicates significant
 561 differences in k between litter types within each habitat and the capital letters indicate
 562 significant differences in k between habitats within each litter type ($p < 0.05$).

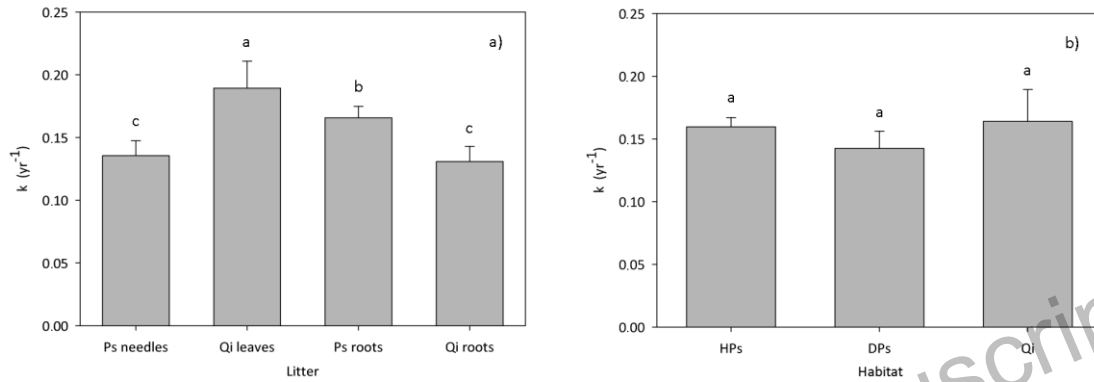


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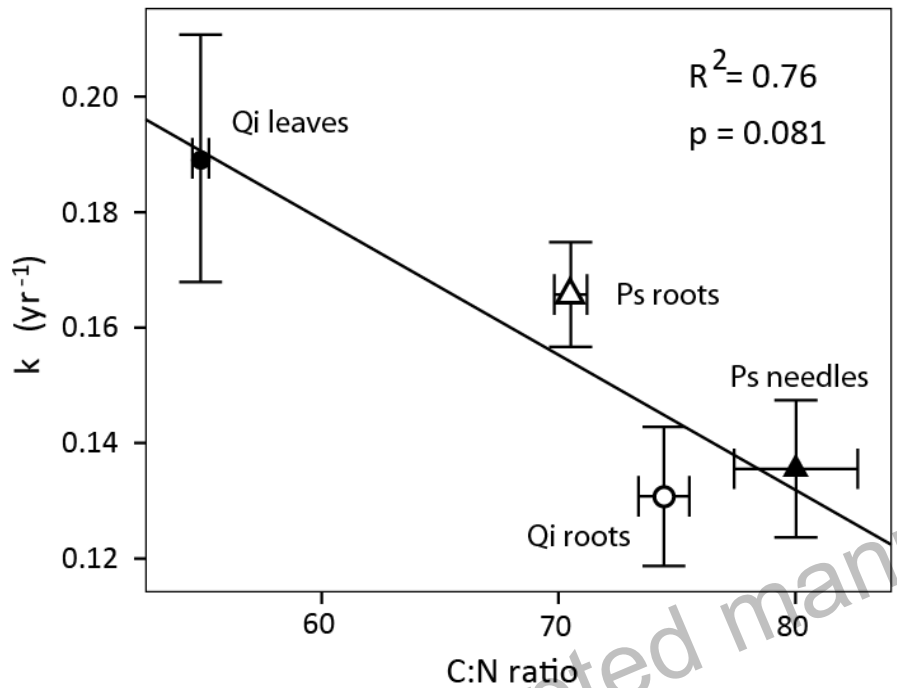
566 **Fig. 2.** Effects of litter type (a) and habitat (b) on the decomposition constant (k) (mean \pm SE)
567 obtained by the GLM. The lower case indicates significant differences in k between litter types
568 (a) and habitats (b) ($p < 0.05$). Significant differences between litter types and habitat types
569 were obtained from GLMs that considered only the respective variables as fixed factors (see
570 text).



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573 **Fig. 3.** Relationship between the initial C:N ratio of litter (leaves and fine roots from *Pinus*
574 *sylvestris* and *Quercus ilex*) and decomposition constant (k) (mean \pm SE), calculated as the
575 mean of k considering the three habitat types.



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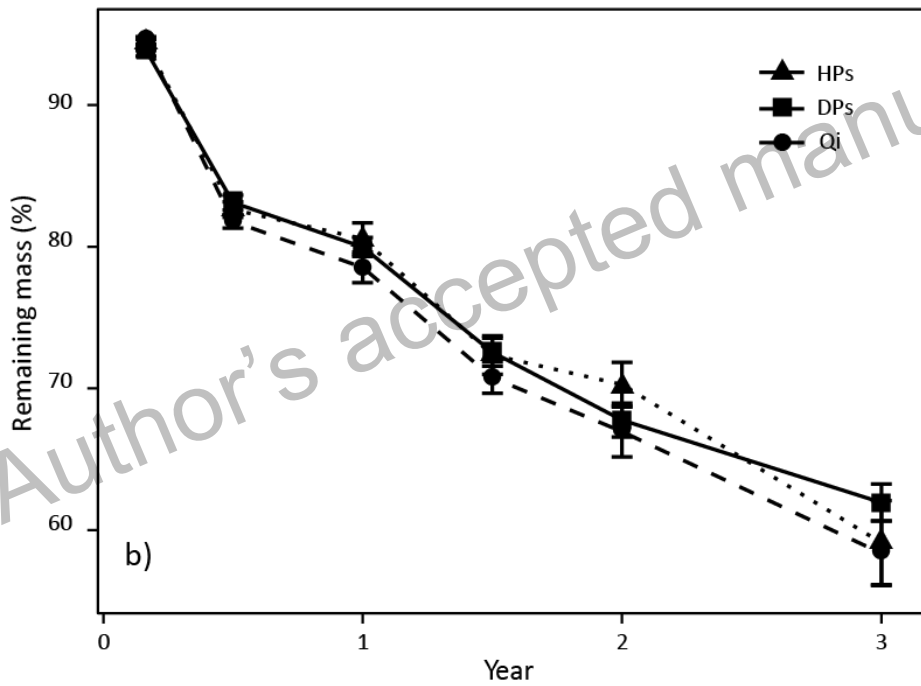
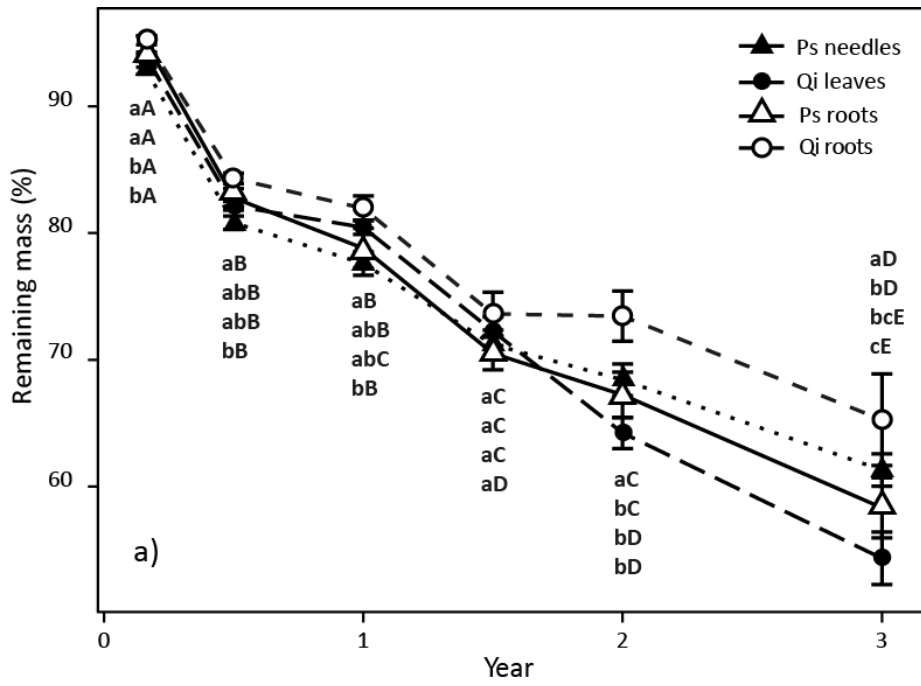
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579 **Fig. 4.** Mass remaining (mean \pm SE) for each litter type (a) and habitat type (b) at 6 collection
580 times (0.16, 0.5, 1, 1.5, 2 and 3 years, n=5). The lower case indicates significant differences in
581 remaining mass between litter types (panel a) within collection times. The capital letters
582 indicate significant differences in remaining mass significant over time within litter types
583 (panel a) and within habitat types (panel b). No significant differences were found in remaining
584 mass between microsites within collection times (b). Comparisons were made with GLMs, with
585 microsite as a random factor ($p < 0.05$). Statistical analyses were performed with remaining
586 mass log-odd transformed to achieve normality.

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589 **Tables**

Variables	df	F-value	p-value
Litter	3	7.701	<0.001
Habitat	6	1.753	0.175
Litter X Habitat	6	2.244	0.039

590

591 **Table1.** Results of the general linear model (GLM) testing for the effects of litter and microsite,
 592 and their interaction, on the decomposition constant (Yr^{-1}).

593

594

595

	Ps needles	Qi leaves	Ps roots	Qi roots
C (%)	52.51 ^a ± 0.50	50.79 ^b ± 0.11	48.62 ^c ± 0.17	48.20 ^c ± 0.31
N (%)	0.67 ^b ± 0.02	0.93 ^a ± 0.01	0.69 ^b ± 0.01	0.65 ^b ± 0.01
C:N	80.02 ^a ± 2.61	54.90 ^c ± 0.35	70.16 ^b ± 0.96	74.45 ^{ab} ± 1.08

596

597 **Table 2.** Initial litter quality on the study site. The different letters indicate differences from
 598 litter from the post-hoc analysis (Tukey test) ($P < 0.05$).