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Elena Ormeño, Julien Ruffault, Caroline Gutigny, Javier Madrigal, Mercedes Guijarro, et al.. Increasing cuticular wax concentrations in a drier climate promote litter flammability. *Forest Ecology and Management*, 2020, 473, pp.118242. 10.1016/j.foreco.2020.118242 . hal-02867824

HAL Id: hal-02867824

<https://amu.hal.science/hal-02867824v1>

Submitted on 16 Jun 2020

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1 **Increasing cuticular wax concentrations in a drier climate promote litter flammability**

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17 **Abstract**

18 Several plant chemical traits (cellulose, tannins and terpenes) have been related to plant
19 flammability. Contrastingly, no study has focused on the relationship between plant
20 flammability and physico-chemical leaf litter traits with a focus on cuticular wax concentration.
21 This study focuses on alkane cuticular waxes because of their relatively low flash point and
22 storage in the cuticle of all vascular plant species. The sclerophyllous species *Quercus coccifera*
23 is the model species since it is the main shrub species in the Mediterranean basin and all
24 previously investigated sclerophyllous species feature a high cuticular wax content. Litter was
25 collected in a Mediterranean garrigue where *Q. coccifera* grows under natural drought and
26 recurrent aggravated drought (consisting of 5.5 years of rain restriction). These different
27 drought conditions were expected to imply different alkane wax concentrations since one of the
28 major roles of cuticular waxes is evapotranspiration limitation during drought. Litter
29 flammability was assessed through ignition delay, flame residence time and flame height
30 (assessed using an epradiator) and gross heat of combustion (using an adiabatic bomb
31 calorimeter). Results showed that the higher cuticular alkane concentrations reached under
32 aggravated drought were associated with an increased leaf litter flammability as expected.
33 These results confirm that all potentially flammable organic metabolites (terpenes as previously
34 reported in other studies, and cuticular alkane waxes) are drivers of vegetation flammability. It
35 is suggested that *Q. coccifera* flammability (considered as low to moderate), could increase
36 under a drier scenario in the Mediterranean area. We hypothesize that fire severity would
37 accordingly be intensified in shrubs dominated by this sclerophyllous species without
38 necessarily increasing vulnerability of *Q. coccifera* to fire since this is a resprouter species after
39 fire and is one of the main pioneer species during post-fire vegetation succession.

40 **Keywords:** ignitability, alkanes, plant metabolites, sclerophyllous species, kermes oak,
41 *Quercus coccifera*, climate change, Mediterranean climate, fire hazard, laboratory tests.

42 **1 Introduction**

43 Mediterranean climate favors wildfire in Mediterranean shrub and forest ecosystems,
44 especially in summer when low precipitation and high temperatures reduce biomass water
45 content (Fares et al., 2017). Projection studies indicate increasing fire danger and frequency in
46 the Mediterranean region because of the drier conditions expected in this region by the end of
47 the century (Moriondo et al., 2006; Turco et al., 2018). Drought severity especially affects fire
48 prone ecosystems, such as Mediterranean garrigues, with vegetation being the only factor
49 influencing fire regime that can be managed to reduce probability of fire events. Proper
50 understanding of fuel traits that are modified as drought is intensified, and their eventual impact
51 on garrigue fire hazard is especially crucial since Mediterranean garrigues are prone to
52 widespread crown fires (D'Odorico et al., 2019) and are one of the most abundant fuel types
53 both in natural and Wildland-Urban Interfaces where garrigues are often juxtaposed with large
54 metropolitan centers (Pimont et al., 2018; Lampin-Maillet et al., 2010). Management of these
55 Mediterranean ecosystems is thus a major challenge of special concern.

56 External factors to the plant (e.g. wind, temperature, air humidity, precipitation,
57 topography, forest management practices) are tightly related to wildfire activity. Internal
58 vegetation factors including physical and chemical traits of green leaves and leaf litter are also
59 drivers of wildfire through their effect on flammability (Varner et al., 2015). Several studies
60 have revealed a clear positive correlation between terpene content and flammability, both in
61 green leaves and litter of Mediterranean plant species (Ormeño et al., 2009; Pausas et al., 2015;
62 Della Rocca et al., 2017). In species with high terpene amounts in specific storage structures
63 like trichomes (*Rosmarinus* spp., *Lavandula* spp.), and resinous cavities (coniferous species),
64 terpene content thereby plays a significant role in plant flammability at least under low fuel
65 water content.

66 The importance of terpene content in flammability of green or dead leaves in litter is
67 however only relevant in terpene storing species. All vascular species possess waxy compounds
68 in the upper cuticle layer (epicuticle) whose potential role in vegetation flammability could be
69 noticeable. Epicuticular waxes cover the leaf forming a lipophilic layer which play numerous
70 ecophysiological roles including evapotranspiration regulation, limitation of herbivore damage
71 (e.g. anti-climb surface) and increasing UV reflection (Shepherd and Wynne Griffiths, 2006).
72 Both, cuticle production and epicuticular wax accumulation can thus be naturally reinforced in
73 plant species to increase tolerance to water deficit for example (Kosma et al., 2009; Xue et al.,
74 2017). The composition of the epicuticle is a complex mixture of long-chain aliphatics,
75 commonly named waxes, most of which can be classified as alkanes, alkanols, alkanals,
76 alkanones, alkanolic acids and alkyl esters. Among all these compounds, alkanes are considered
77 as the only ubiquitous waxes since they are present in the cuticle of almost all vascular plant
78 species (Heredia and Dominguez, 2009). These compounds, which mostly range from C₂₁ to
79 C₃₅, are also of particular interest due to their influence on flammability. The flash point, the
80 temperature at which a liquid fuel gives off sufficient vapor resulting in a flash fire when an
81 ignition source is present, ranges from 120 to 356 °C respectively (as reported in several
82 chemical manufacturer's Material Safety Data Sheets). Based on the relatively high
83 flammability of alkane-waxes and the potential role of waxes to limit water losses in plants, it
84 is important to evaluate whether aggravated drought expected under Mediterranean climate
85 change can lead to an increased accumulation of alkane-waxes in green or litter leaves
86 eventually favoring their flammability.

87 It deserves special attention to evaluate traits that influence litter flammability since this
88 fuel is ubiquitously present in all vegetation formations, fires often start in litter and fire
89 behavior is influenced by surface fuels and senesced leaf litter in particular (Plucinski and
90 Anderson, 2008; Varner et al., 2015). Moreover, litter provides relatively extensive surface

91 loads of fuels with poor moisture content in which individual leaves are easy to ignite and thus
92 contribute to the initial fire propagation (Plucinski and Anderson, 2008). Then, well-connected
93 surface fuels enable fire to spread easier since they influence its transmission to the upper
94 vegetation (Belcher, 2016).

95 The importance of litter as a fuel could be especially relevant in dense *Quercus coccifera*
96 L. shrubs under aggravated drought since sclerophyllous species features both, high annual litter
97 production ($968.8 \pm 43.8 \text{ g m}^{-2}$) compared to other shrub species (e.g. *Rosmarinus officinalis*
98 L., and *Ulex parviflorus* Pourr) (Rodriguez-Ramirez et al., 2017) and slow decomposition rates,
99 especially under aggravated drought conditions when fuel moisture content is the lowest
100 (Santonja et al., 2019). Thus, this shrub species thereby provides a relatively high annual
101 amount of dead particles that accumulate on the soil. Moreover, *Q. coccifera* accounts for the
102 main shrub species in Southern France where it occupies more than 100 000 ha and more than
103 2 million ha in the Mediterranean region (Le Hou  rou, 1973; Trabaud, 1979). Although *Q.*
104 *coccifera* litter features low ignitability as tested in laboratory studies using litter beds (Curt et
105 al., 2010), epicuticular waxes (never documented so far) could be highly concentrated in a more
106 arid climate eventually promoting litter flammability. Indeed, cuticular waxes are highly
107 concentrated in leaves of other sclerophyllous and evergreen Mediterranean species (*Olea*
108 *europaea* L., *Quercus suber* L., and *Quercus ilex* L.) (Martins et al., 1999; Huang et al., 2017).

109 The main aim of this study was to test in the laboratory the relationship between *Q.*
110 *coccifera* leaf litter flammability and epicuticular alkane concentrations, through a range of
111 alkane concentrations expected to occur under natural and recurrent aggravated drought
112 conditions simulated in the field with rain exclusion systems. We additionally evaluated the
113 relationship between litter flammability and several physical traits including litter cuticle
114 production and the classical traits, leaf area and specific leaf area (SLA). Anticipating if a drier
115 climate will modify physico-chemical litter traits in sclerophyllous evergreen species and

116 consequences on litter flammability is of special interest in fire ecology and fire management
117 in garrigue ecosystems.

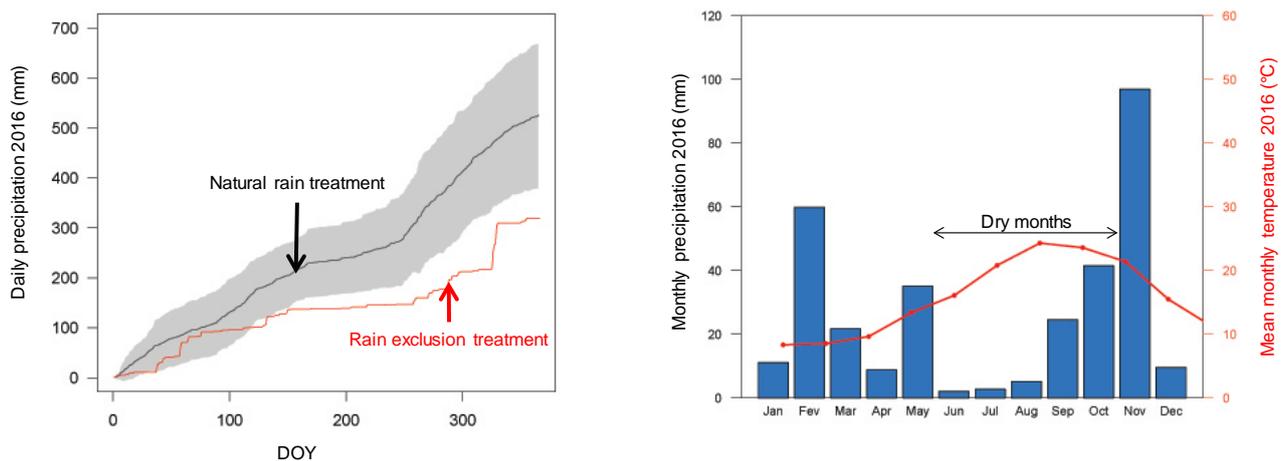
118 **2 Materials and methods**

119 *2.1 Experimental site and design*

120 The study was carried out in the experimental site of CLIMED ([https://www.imbe.fr/zoom-sur-](https://www.imbe.fr/zoom-sur-l-anr-climed.html)
121 [l-anr-climed.html](https://www.imbe.fr/zoom-sur-l-anr-climed.html)), a garrigue where *Q. coccifera* (Fagaceae) is the dominant shrub species.
122 Other species co-exist including the perennial grass *Brachypodium retusum* P. Beauv.
123 (Poaceae) and the shrubs *Cistus albidus* L. (Cistaceae), *Rosmarinus officinalis* L. (Lamiaceae)
124 and *Ulex parviflorus* Pourr. (Fabaceae). The site is located at the Chaîne de l'Etoile mountain
125 in the North of Marseille (43°220 N; 5°250 E, south France, 275 m a.s.l., mean slope < 1°). For
126 soil details of the site see Rodriguez Ramirez et al. (2017). The site features a Mediterranean
127 climate with hot and dry summers (on average over 30 years, 100 days y⁻¹) and wind throughout
128 the year with a minimum average wind speed of 57 km h⁻¹ (highly windy site,
129 www.infoclimat.fr). Mean annual temperature at the site is 14.6 °C and mean annual
130 precipitation is 552 mm averaged over the period 2002 - 2015 from the two closest
131 meteorological stations to our experimental site (Météo France stations: Marignane 43°260 N,
132 5°120 E and Marseille 43°150 N, 5°220 E). Total annual precipitation during 2016 (the year
133 preceding litter sampling) was 530 mm (Fig. 1), in the range of the mean annual precipitation
134 recorded between 2011 and 2015 (www.infoclimat.fr). The last fire in this *Q. coccifera* garrigue
135 occurred in 1997 when 3,500 ha burned from a total of 10,000 ha garrigue.

136 Rain exclusion (recurrent aggravated drought treatment) was carried out in 10 rain plots
137 set-up in October 2011 that received on average 30 % less rainfall compared to control plots
138 (Fig. 1). Each rain exclusion plot presented 16 m² surface and was equipped with a 4 m × 4 m
139 solid aluminum frame held 2 m above the ground, using aluminum posts at the outer

140 circumference of the 16 m² plot area and fixed to the ground with reinforcing bars. Stainless
 141 steel gutters were mounted on top of the aluminum frame. A supplementary PVC gutter and a
 142 pipe mounted at the border of the frame allows for evacuation of rainwater away from the plots.
 143 The control plots consisted of 10 other plots (16 m², 4 m × 4 m) adjacent to the rain exclusion
 144 plots. Control plots received 100% rain since they did not present any solid frame above the
 145 ground. Leaf litter of *Q. coccifera* was collected in the 4-m² central zone of each plot to avoid
 146 edge effect. In all plots (rain exclusion and control plots), *Q. coccifera* was the major species
 147 with at least 80 % of *Q. coccifera* cover. Litter from the 20 plots was collected in February
 148 2017, that is, 5.5 years after rain exclusion was applied. Only superficial and entire leaf litter
 149 was collected, corresponding to litter from the first stages of decomposition.



150
 151 **Fig. 1** Cumulative precipitation (left graph) and ombrothermic diagram (right graph) for the
 152 experimental site (CLIMED) in 2016, before litter was collected (February 2017). For
 153 cumulative precipitation, mean (line) and standard deviation (shaded area) are represented.

154 2.2 Litter conditioning before physico-chemical analysis

155 Leaf litter was partially dehydrated before any chemical or flammability analysis. This
 156 approach resulted in an average litter moisture content just below 15 % (13.2 ± 0.4 and $13.0 \pm$
 157 0.5 , mean \pm standard error, under natural and recurrent aggravated drought respectively), since
 158 litter is extremely flammable below this threshold (Whelan, 1995; de Dios et al 2015). Litter
 159 was however not completely dehydrated to work under realistic litter moisture contents during
 160 summer months (Larchevêque et al 2005, data from CLIMED). Partial dehydration was

161 achieved by oven-drying leaf litter at 35 °C during 4 days. Litter moisture content was
162 calculated as follows:

$$163 \quad \text{Litter humidity after partial dehydration (\%)} = \frac{\text{Partially dried mass} - \text{Dry mass}}{\text{Dry mass}} \times 100$$

164 where dry mass corresponds to oven dried litter mass at 60 °C during 72 h, and partially dried
165 mass corresponds to oven-dried litter mass at 35 °C during 4 days. Within each plot, three
166 samples of 5 g each were used to calculate litter moisture content after partial dehydration.
167 These samples were not used for cuticular alkane extractions to avoid potential losses of these
168 waxes during the oven-drying process.

169 *2.3 Flammability measurements*

170 The following flammability parameters were determined on partially dehydrated litter: *i*)
171 ignition delay (ignitability indicator) measured as the time flame took to appear since litter was
172 placed in a epiradiator (see below) and expressed in s, *ii*) flame residence time or duration of
173 flaming combustion (sustainability indicator) measured as the time flame was visible from
174 ignition until extinction and expressed in s, *iii*) maximum flame height (combustibility
175 indicator) measured in cm according to a reference grid, and, *iv*) gross heat of combustion
176 produced during complete combustion (sustainability indicator) expressed in KJ g⁻¹.

177 Ignition delay, flame residence time and maximum flame height were measured in a
178 closed room using a standard epiradiator delivering a 500 W constant nominal power rating.
179 The epiradiator had a surface made of a vitreous fused silica disk of 100 mm diameter
180 generating a surface standard temperature of 420 °C. Flammability was measured on 10 pseudo-
181 replicates of leaf litter from each rain exclusion plot and each control plot (totaling 195
182 measurements since 5 measurements were technically lost). To this purpose, 0.5 g of partially
183 dried litter mass (corresponding to 13 – 25 leaves) was put in the center of the silica disk.

184 Gross heat of combustion was determined following the method outlined in the
185 International Standard ISO 1716 (Madrigal et al., 2011). Within each plot, a representative litter
186 sample was collected and ground to 0.51 mm in a mill. Pellets of about 1 g of the ground
187 material were prepared using a hand press, oven-dried at 60 °C for 48 h and then weighed.
188 Measurements were made with an adiabatic bomb calorimeter equipped with a platinum
189 resistance sensor (PT-100). Both, mill and adiabatic bomb calorimeter were manufactured by
190 IKA®. Gross heat of combustion of each plot was obtained as the average of the measurements
191 obtained on three replicates per plot which comply with the repeatability criteria (standard error
192 less than 1 %).

193 *2.4 Epicuticular alkane extraction and analysis*

194 Epicuticular wax extraction did not aim to be exhaustive but was focused on alkane extraction
195 alone. For this purpose, 1 g of partially dried litter was immersed into 6 ml of an organic solvent
196 mixture formed by cyclohexane and chloroform (70:30 volume) containing a fixed amount
197 (37.1 ng) of an internal standard (IS, undecane). This IS was not naturally present in litter
198 leaves. Within each plot, we performed three extractions from the same litter pool (totaling 60
199 extractions). Extractions were performed with constant shaking for 60 s. The extract was
200 filtered with a PTFE syringe filter (0.2 µm, 30 mm diameter). Analyses were performed using
201 a gas chromatograph (GC, Hewlett Packard GC6890®) coupled to a mass spectrometer (MS,
202 HP 5973N). The HP-5MS capillary column (30 m) in the GC was in constant flow mode and
203 was connected directly to the MS. Sampled volumes (1 µl) of each alkane extract were injected
204 through an automatic injector (ALS 7683). Helium (99.995 %) was used as carrier gas. The
205 oven temperature was initially set at 50 °C and then it increased to 160 °C at a rate of 2 °C min⁻¹.
206 It then remained constant for 5 min. Epicuticular alkanes were identified by comparison of
207 the retention time and mass spectrum of detected compounds with those of the authentic
208 reference samples (n-C₂₁-C₄₀ alkane standard from Aldrich–Firmenich). Their identity was then

209 confirmed with generated libraries of retention indexes (Adams 2007). Alkanes were quantified
210 by considering their relative response factor (RRF) using the following formula:

$$\text{RRF} = \frac{S_{\text{Alkane}}/S_{\text{IS}}}{Q_{\text{Alkane}}/Q_{\text{IS}}}$$

211
212 where S_{Alkane} is the chromatographic surface of the detected alkane, S_{IS} is the chromatographic
213 surface of the internal standard, Q_{Alkane} is the amount of the detected cuticular compound that
214 needs to be quantified, and Q_{IS} is the known and fixed amount of the internal standard. The n-
215 $\text{C}_{21}\text{-C}_{40}$ alkane standard with known concentration was used for calculating RRF of each alkane.
216 The extracted alkanes were also expressed as the percentage they represented within the total
217 cuticle (see cuticle extraction method hereafter).

218 *2.5 Total cuticle extraction*

219 Litter cuticle was extracted using a fixed volume (1.5 ml) of the previous organic solvent
220 mixture (cyclohexane and chloroform, 70:30 volume). This volume was added to 1 g of partially
221 dried leaf litter. After full solvent evaporation using a Stuart concentrator SBHCONC/1
222 connected to a nitrogen flow, cuticle mass was gravimetrically calculated as the mass difference
223 between the vial containing the cuticle and the vial alone. Cuticle production was expressed in
224 $\text{mg}_{\text{cuticle}} \text{g}_{\text{DM}}^{-1}$ and relative cuticle production was expressed in percentage and calculated as
225 $[(100 \times \text{litter cuticle mass})/\text{litter dry mass}]$. Cuticle extraction was measured in 3 pseudo-
226 replicates within each plot.

227 *2.6 Leaf area and specific leaf area measurements*

228 SLA ($\text{cm}^2 \text{g}_{\text{DM}}^{-1}$) was calculated on 45 leaves within each plot. The one-sided surface area of
229 all the leaves considered together was calculated with the computing program *WinSeedle*. SLA
230 was calculated by dividing this total leaf litter area by the corresponding total dry mass
231 (obtained by oven-drying for 72 h). Single leaf area was measured using the same program.

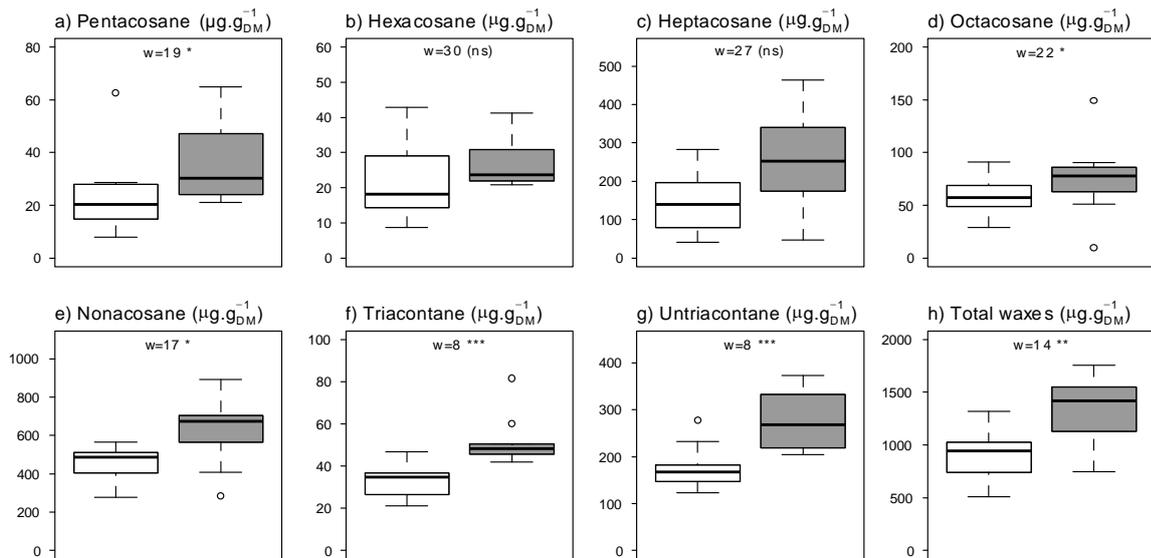
232 2.7 Statistical analysis

233 To test the effect of drought treatment on physico-chemical traits and flammability parameters
234 of leaf litter, we used Mann-Whitney tests. Two Principal Component Analysis (PCA) followed
235 by PERMANOVA tests were performed to check for differences between litter from natural
236 and aggravated recurrent drought in terms of flammability metrics on the one hand, and in terms
237 of physico-chemical variables on the other hand. Scores from the physico-chemical variables
238 PCA (x axis) were then correlated to scores from flammability metrics PCA (y axis) to evaluate
239 the relationship between these two dimensions. Pearson's correlations and Pearson matrices
240 were used to evaluate the association between physico-chemical traits and flammability
241 parameters after log transformation of the data. All statistical analyses were carried out using
242 the program *R* (*ade4* package for PCA).

243 3 Results

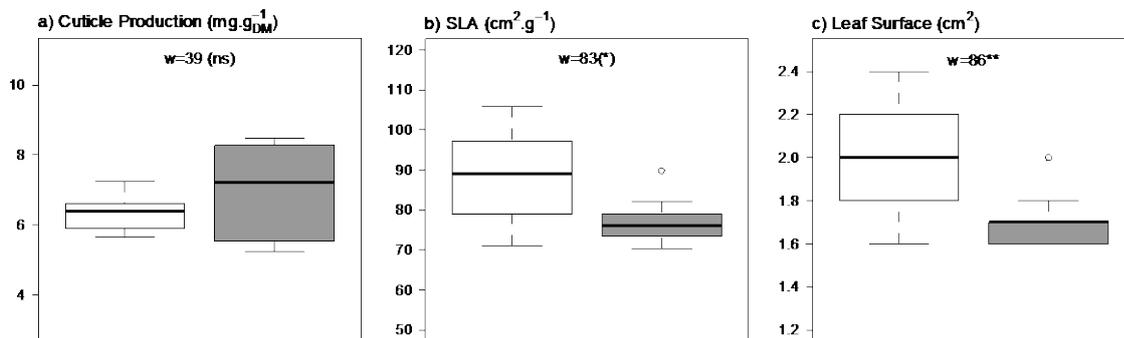
244 3.1. Litter physico-chemical traits under drought treatments

245 Alkanes extracted from epicuticle of *Q. coccifera* litter included pentacosane ($C_{25}H_{48}$),
246 hexacosane ($C_{26}H_{50}$), heptacosane ($C_{27}H_{52}$), octacosane ($C_{28}H_{54}$), nonacosane ($C_{29}H_{56}$),
247 triacontane ($C_{30}H_{58}$) and untriacontane ($C_{31}H_{60}$). The major alkane was C_{29} (573.0 ± 33.7 ng
248 DM^{-1}), followed by C_{31} (240.4 ± 13.9 ng g_{DM}^{-1}) and C_{27} (208.8 ± 19.7 ng g_{DM}^{-1}) (Fig. 2).
249 Concentrations of alkanes showed highly significant correlations (Table S1). Total
250 concentration of epicuticular alkanes in litter of *Q. coccifera* was 1189.4 ± 68.0 μg g_{DM}^{-1}
251 (representing 18.6 ± 1 % of the cuticle mass) or 14.8 ± 0.9 μg cm^{-2} on average. It was
252 significantly higher (+ 50%) under aggravated drought (1458 μg g_{DM}^{-1}) compared to natural
253 drought (920 μg g_{DM}^{-1}) (Fig.2h). This difference was due to C_{25} , C_{28} , C_{29} , C_{30} and C_{31} whose
254 concentration was significantly promoted under aggravated drought (Fig. 2a, d, e, f, g).



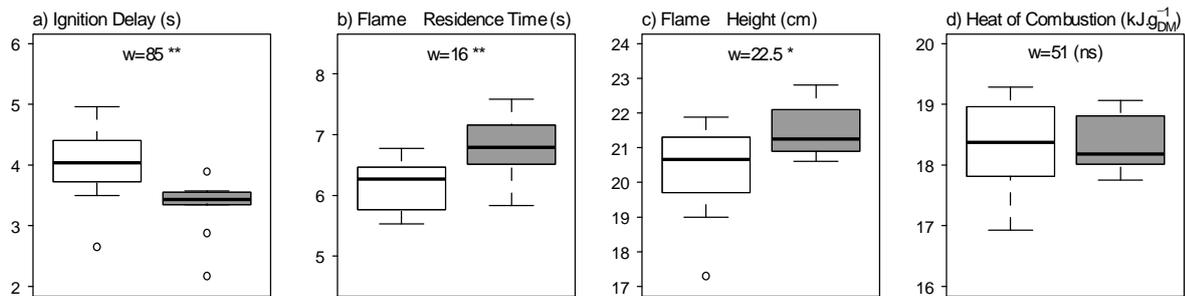
255
 256 **Fig. 2** Concentration ($\mu\text{g g}_{\text{DM}}^{-1}$) of n-alkanes in litter epicuticle of *Q. coccifera* under natural
 257 (white) and aggravated (grey) drought. Differences between both treatments are tested with
 258 Mann-Whitney tests (w). The central line is the median, the edges of the box extent from 1st
 259 quartile to 3rd quartile (25-75 %) and the limits of the whiskers extent to the 1.5 interquartile
 260 range. Points indicate outliers. *: $0.01 < P < 0.05$; ***: $P < 0.001$; ns: not significant (n=10).

261 Among physical traits, cuticle production was on average $6.7 \pm 0.22 \text{ mg g}_{\text{DM}}^{-1}$ (or 82.2
 262 $\pm 4.1 \mu\text{g cm}^{-2}$). Cuticle production was not significantly different between treatments (Fig. 3a).
 263 The average SLA value was $82 \pm 2.3 \text{ cm}^2 \text{ g}_{\text{DM}}^{-1}$ with a marginal drop of SLA under aggravated
 264 drought ($76 \text{ cm}^2 \text{ g}_{\text{DM}}^{-1}$) compared to litter from natural drought ($88.5 \text{ cm}^2 \text{ g}_{\text{DM}}^{-1}$, Fig. 3b). This
 265 difference is associated to the smaller surface of leaf litter (-13 %) under aggravated drought
 266 compared to natural drought (Fig. 3c).



267
 268 **Fig. 3** Leaf litter physical traits under natural (white) and aggravated (grey) drought conditions.
 269 The central line is the median, the edges of the box extent from 1st quartile to 3rd
 270 quartile (25-75 %) and the limits of the whiskers extent to the 1.5 interquartile range. Significant differences
 271 are tested with U-Mann-Whitney tests (w: value of the test). Points indicate outliers. (*):
 272 $0.05 < P < 0.10$ (marginal significance); **: $0.001 < P < 0.01$; ns: not significant (n=10).

273 3.2. Litter flammability: variability with drought and correlations with physico-chemical traits
 274 Litter from the aggravated drought treatment exhibited a shorter ignition delay, longer flame
 275 residence time and higher maximum flame height (Fig. 4a-c, $P < 0.05$) compared to litter from
 276 the natural drought treatment. No effect of drought treatment was detected for gross heat of
 277 combustion (Fig. 4d).



278 **Fig. 4** Leaf litter flammability when litter originates from natural (white) and aggravated (grey)
 279 drought conditions. The central line is the median, the edges of the box extent from 1st quartile
 280 to 3rd quartile (25-75 %) and the limits of the whiskers extent to the 1.5 interquartile range.
 281 Points indicate outliers. *: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$ ns: not significant (n=10).
 282

283 Ignition delay and gross heat of combustion were the main flammability parameters that
 284 correlated to litter physico-chemical traits (Table 1). Ignition delay correlated negatively with
 285 all alkanes (excepting heptacosane content) (Table 1), indicating that alkane accumulation
 286 shortened this delay. The best correlations (Fig. 5) occurred with pentacosane, hexacosane,
 287 nonacosane, triacontane and the total alkane concentration. Gross heat of combustion rose with
 288 increasing amounts of nonacosane, the major alkane found in leaf litter cuticle. The same result
 289 was shown for octacosane, and to a lesser extent, hexacosane, nonacosane and total alkane
 290 content. When alkanes were expressed as the percentage they represented in the cuticle, this
 291 relative alkane concentration also correlated to gross heat of combustion although only
 292 marginally (Table 1). By contrast, none of the studied flammability parameters was correlated
 293 to cuticle production (Table 1). Flame residence time (or combustion duration) only increased
 294 significantly with heptacosane, one of the major alkanes (Fig. 5), although a marginal increase

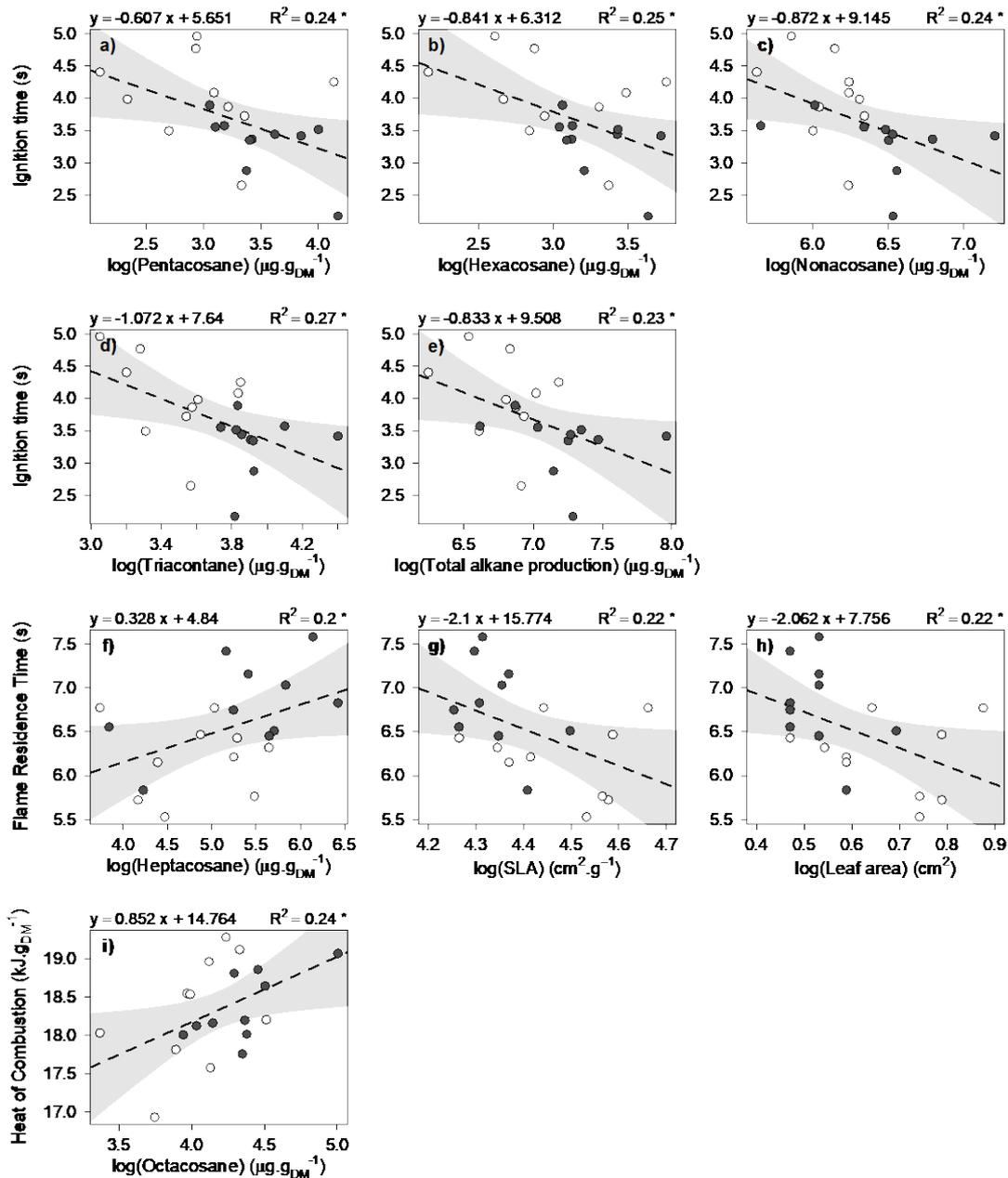
295 also occurred with pentacosane and triacontane (minor alkanes) (Table 1). Maximum flame
 296 height marginally correlated to triacontane content alone (Table 1).

297 **Table 1** Correlation matrix between physico-chemical traits and flammability metrics showing
 298 Pearson's correlation coefficient (r) and significance of their linear relationship. Data are log-
 299 transformed to achieve a normal distribution.

Flammability parameter	Ignition delay (s)	Flame Residence Time (s)	Maximum flame Height (cm)	Gross heat of combustion (KJ g _{DM} ⁻¹)
Physico-chemical trait				
Pentacosane (μg g _{DM} ⁻¹)	-0.49*	0.41(*)	0.13	0.15
Hexacosane (μg g _{DM} ⁻¹)	-0.50*	0.34	0.31	0.42(*)
Heptacosane (μg.g _{DM} ⁻¹)	-0.26	0.44*	0.11	0.33
Octacosane (μg g _{DM} ⁻¹)	-0.44(*)	0.35	0.27	0.49*
Nonacosane (μg g _{DM} ⁻¹)	-0.49*	0.28	0.27	0.44(*)
Triacontane (μg g _{DM} ⁻¹)	-0.52*	0.39(*)	0.50*	0.40(*)
Untriacontane (μg g _{DM} ⁻¹)	-0.39(*)	0.28	0.31	0.33
Total alkane production (μg g _{DM} ⁻¹)	-0.48*	0.38(*)	0.28	0.44(*)
Relative alkane concentration (%)	-0.34(*)	0.30	0.35	0.43(*)
Cuticle production (mg g _{DM} ⁻¹)	-0.26	0.26	-0.07	-0.06
SLA (cm ² g _{DM} ⁻¹)	0.05	-0.45*	-0.22	0.02
Leaf area (cm ²)	0.19	-0.45*	-0.24	-0.004

300 (*): 0.05 < P < 0.1; *: 0.01 < P < 0.05; ns: not significant (P > 0.10); n=20.

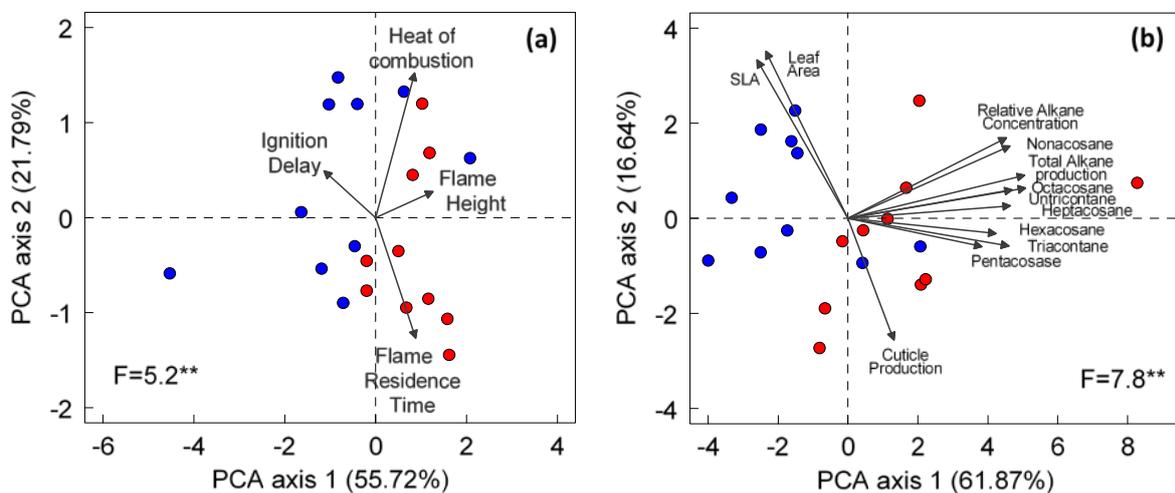
302
 303 Flammability parameters were explained through 2 axes, where PCA1 captured 56% of
 304 the dataset variability and mainly explained differences in terms of ignition delay and maximal
 305 flame height, while PCA2 covered 22 % of the data variability and was strongly explained by
 306 heat of combustion and flame residence time. Flammability parameters were highly associated
 307 (P < 0.001, Table S2, Fig. 6a) with a tight negative correlation between ignition delay and both,
 308 flame residence time and maximum flame height (P < 0.001). These two later flammability
 309 parameters were positively correlated, indicating that when flame sustainability increased
 310 combustibility increased too. Gross heat of combustion was significantly correlated with
 311 maximum flame height.



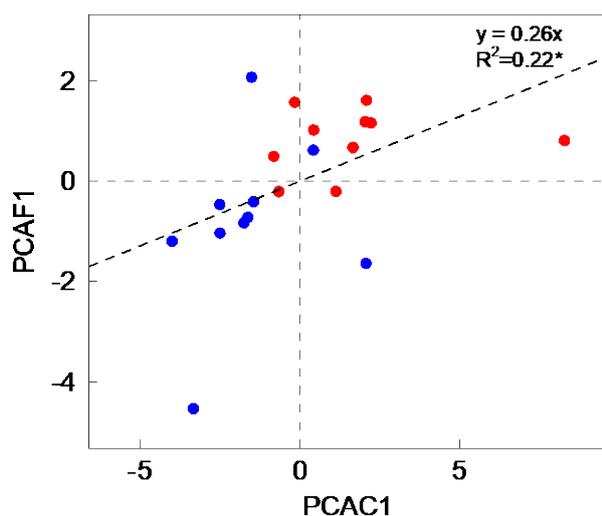
312

313 **Fig. 5** Pearson correlations between physico-chemical traits (x axis) and flammability
 314 parameters using log-transformed data (y axis). Black and white points refer to natural and
 315 aggravated drought treatments respectively. Points represent the average \pm SE values (n=10).
 316 Shaded grey represents the prediction limits of the models. Only significant correlations (with
 317 $P < 0.05$) from Table 1 are shown.

318 Multivariate analysis allowed to have an overall view of the differences between
 319 aggravated drought and natural drought in terms of flammability (Fig. 6a) and, physico-
 320 chemical litter traits (epicuticular alkanes, SLA, leaf area, cuticle production) (Fig. 6b). Litter
 321 from aggravated drought showed a shorter ignition delay, a longer flame height (PCA1) and a
 322 longer flame residence time (PCA2) (Fig. 6a), higher alkane content, smaller leaf area, lower
 323 SLA under aggravated drought. Previous non parametric tests revealed these differences were
 324 significant. Scores from both PCAs were positively and linearly correlated (Fig. 7) indicating
 325 that the differences in flammability were driven by litter physico-chemical traits.



326
 327 **Fig. 6.** Principal Component Analysis showing differences between natural (●) and aggravated
 328 drought (●) in terms of (a) leaf flammability metrics (maximal flame height and ignition delay
 329 explain axis 1 and gross heat of combustion and flame residence explain axis 2) and, (b)
 330 physico-chemical variables (most explanatory variables are alkanes from C₂₇ to C₃₁ and total
 331 alkane content in axis 1 and SLA, leaf area and cuticle production in axis 2). Percentages refer
 332 to variability explained by each axis. PERMANOVA tests (F) followed by ** denote the
 333 significance of the differences between the treatments (**: 0.01 < P < 0.001). The axes scores of
 334 each variable are provided in Table S3.



335

336 **Fig. 7.** Correlation between the axis scores resulting from the physico-chemical litter traits
 337 based-PCA (x axis) (obtained in Fig. 6a) and axis scores resulting from the leaf flammability
 338 based-PCA (y axis) (obtained in Fig. 6b).

339 **4 Discussion**

340 This study provides information about physico-chemical leaf litter changes during drought and
 341 their eventual influence on flammability, with novel information being related to alkane
 342 content.

343 *4.1 Chemical and physical trait influence on leaf litter flammability*

344 Most alkanes in litter cuticle of *Q. coccifera* presented an odd number of carbon atoms, as
 345 commonly shown in other species (Diefendorf et al., 2015). Nonacosane (C₂₉) was the major
 346 compound in accordance with the epicuticular alkane composition found in *Q. ilex*, *Q. suber*
 347 and *Q. calliprinos* (Palestine oak) (Martins et al., 1999; Brosh et al., 2003; Norstrom et al.,
 348 2017). Wax concentration and coverage in leaves is extremely variable among species, from
 349 0.4 (minimum wax coverage) to 160 $\mu\text{g cm}^{-2}$ (maximum wax coverage) (Deininger, 2016). In
 350 view of our results, alkane concentration in leaf litter of *Q. coccifera* (1189.4 $\mu\text{g g}_{\text{DM}}^{-1}$ or 14.8
 351 $\mu\text{g cm}^{-2}$) can thereby be considered a major alkane cover, in the range of those found in cuticles
 352 of green leaves of other Mediterranean sclerophyllous species like *Q. suber* (18 $\mu\text{g cm}^{-2}$), *Q.*
 353 *ilex* (7 $\mu\text{g cm}^{-2}$) (Martins et al., 1999) and *O. europaea* (8.5 $\mu\text{g cm}^{-2}$) (Huang et al., 2017), which

354 is well above the alkane concentration in conifer species (from dizaines to less than 900 $\mu\text{g gDM}^{-1}$
355 ¹ (Diefendorf et al., 2015).

356 Ignitability (through ignition delay) was the main litter flammability parameter
357 impacted by litter epicuticular alkane cover. This result suggests that fuels rich in such organic
358 metabolites could become more flammable in the presence of an ignition source since alkane
359 flash points are readily attained in the adjacent vegetation of a wildfire. It is important to note
360 that this relationship between ignitability and wax content was obtained using litter that
361 possessed a natural range of alkane concentrations and a low moisture content (~13 %) under
362 both drought conditions, which is close to the natural range of leaf litter moisture content in the
363 study site during summer (Larchevêque et al., 2005) when flammability of vegetation is the
364 highest. Fuel moisture content is one of the major internal factors determining fuel flammability
365 and fire danger (e.g. Alessio et al., 2008; Pausas et al., 2015). Thus, during an aggravated
366 drought we would expect lower fuel moisture content and thus greater flammability would
367 probably exacerbate differences.

368 Based on the ignition delay of green leaves, *Q. coccifera* is considered a moderately
369 flammable species, alike other species with hard and waxy leaves (e.g. *Cistus* spp., *Pistacia*
370 *lentiscus*), compared to extremely flammable species (*Eucalyptus*) and highly flammable
371 species (e.g. *Pinus* spp., *Q. ilex*, *O. europaea*) and poorly flammable species (e.g. *Juniperus*
372 spp.) (Dimitrakopoulos and Papaioannou, 2001). *Q. coccifera* green leaves are also considered
373 moderately flammable according to its pyric properties (heat content, total and mineral ash
374 content, surface area-to-volume ratio and particle density) (Dimitrakopoulos, 2001). We did not
375 find any study having measured *Q. coccifera* flammability at the leaf scale but when
376 considering litter fuel beds, Curt et al. (2010) reported that dense *Q. coccifera* shrubs forms
377 litters of high bulk density with thick and tough leaves which entail a longer time to ignition
378 and thus low ignitability compared to other shrub species (*Erica* spp.). Our study indicates that

379 aggravated drought could imply a shift towards higher litter ignitability through increases of
380 wax cover.

381 Our work also demonstrates a negative relationship between flame sustainability
382 (through flame residence time) and both, leaf area and SLA as previously shown (Scarff and
383 Westoby, 2006; Grootemaat et al., 2017; Ganteaume, 2018). Correlations with SLA
384 theoretically indicate that in denser and/or thicker leaf tissues (low SLA values) flame
385 sustainability is promoted. Leaf density and thickness were however not studied herein and we
386 can only explain that the lower SLA values (reached in aggravated drought conditions) were
387 associated with low leaf area, which has been suggested as the most important leaf trait in litter
388 flammability (Scarff and Westoby, 2006). Smaller leaves and so presumably shorter leaves may
389 compact leaf litter fuel thereby limiting the permeability of oxygen of the fuel bed and
390 eventually increasing flame residence time (Scarff and Westoby, 2006; de Magalhães and
391 Schwilk, 2012). This can explain why high ignitability (found under aggravated drought where
392 leaf area was smaller) was associated to samples that burned longer (Fig 4).

393 *4.2. Understanding litter trait changes according to water deficit*

394 The high epicuticular alkane concentration in leaf litter under aggravated drought was probably
395 due to the combination of both, limited microbial activity under limited water conditions which
396 slows down the litter decomposing process (Ormeño et al., 2006; Santonja et al., 2015) and
397 plant history, although none of these points were checked in this study. When litter fall occurs,
398 some green leaf traits must be reflected in litter at least during the first stages of decomposition.
399 Epicuticular wax coverage in green leaves fulfills major biological functions in living plants
400 including water conservation (by preventing non-stomatal water loss), nutrient conservation
401 and limiting abiotic and biotic stress-related damage (Shepherd and Wynne Griffiths, 2006;
402 Barthlott et al., 2017). Thus, under water scarcity, wax accumulation in the leaf cuticle often
403 increases (Bacelar et al., 2012). Under aggravated and recurrent drought, *Q. coccifera* litter also

404 exhibited smaller leaves resulting in smaller SLA and so potentially thicker and/or denser
405 leaves. Such responses under aggravated drought (high leaf wax cover, small leaf area, low
406 SLA) are characteristic of sclerophyllous species (Gratani and Varone, 2006) and probably
407 allow *Q. coccifera* to maximize its fitness under scarce water conditions in fire-prone
408 ecosystems. Increasing wax load and reducing leaf area would limit evapotranspiration during
409 long-term drought while implying a higher fire hazard with minimal stand disturbance since
410 post-fire resprouting is the main recolonisation mechanism of *Q. coccifera*. This species
411 possesses the ability to regrow after burning by shoots from stocks and suckers from the roots
412 (Konstantinidis et al., 2005).

413 *4.3. Potential management and ecological implications of these findings*

414 This study investigates the relationship between flammability and physical-chemical fuel
415 traits at a low hydric condition, with the main novel result being the positive influence of
416 vegetation waxes on flammability, never reported so far. Moreover, results highlight that
417 aggravated drought promotes both, ignitability (through accumulation of alkane waxes in
418 cuticle of leaf litter independently of litter water content) and flame sustainability (through the
419 concomitant reduction of leaf area and SLA). We speculate that such relationships could also
420 occur in green leaves of *Q. coccifera* where the higher water content in green leaves compared
421 to leaf litter could be compensated by the high shoot biomass and so the increasing wax content
422 at the stand scale.

423 It is worth noting that epiradiator tests do not pretend to mirror what occurs in the field. In
424 fact, each measurement scale (leaf-scale, fuel bed scale, field burning) provides different
425 information. Epiradiator tests do not integrate fuel structure but are an adequate approach to
426 assess the importance of different leaf traits on some flammability metrics and the best choice
427 for this study since each experimental plot possesses 16 m² surface and only litter from the
428 central 4 m² was collected. Thus, important amounts of litter cannot be collected within each

429 plot impeding the use of leaf litter beds to study flammability. Although burnings performed in
430 the field provide better estimates of fire behavior in a given ecosystem, explaining the factors
431 involved in fire behavior is a complex task due to the influence of multiple biotic factors under
432 natural conditions. For example, in the study of Trabaud (1979) based on burnings of *Q.*
433 *coccifera* in different plots in a garrigue in Southern France, the authors noted that *Q. coccifera*
434 fire behavior and flammability was influenced by the high water content of grasses (co-existing
435 with *Q. coccifera*), which was by far larger compared to woody species.

436 Scaling-up from laboratory to ecosystem measurements is a fundamental point in fire
437 ecology and one of the main challenging steps (Schwilk, 2015; Schwilk and Caprio, 2011;
438 Stevens et al., 2020). Numerous factors (fuel structure, load and mixtures among others) explain
439 fire behavior and hazard in the field. Considering that fire severity (amount of living material
440 consumed) provides a description of how fire intensity (level of heat) affects ecosystems
441 (Keeley, 2009), this study cannot directly provide such information since field experiments
442 were not conducted. We can nevertheless hypothesize that modification of fuel structure and
443 chemistry through modification of litter physical and chemical traits will govern both fire
444 intensity and severity (Belcher, 2016). Moreover, in the few studies that have used similar
445 species in laboratory and field experiments, field evidence supports lab results (Varner et al.,
446 2015). Tumino et al. (2019) also demonstrated that some traits known to influence flammability
447 in the laboratory were associated to field-scale flammability metrics like fire severity. Likewise,
448 Ormeño et al. (2009) (using leaf litter) and Grootemaat et al. (2017) (using green leaves) show
449 that leaf traits influencing flammability of individual leaves continue to do so even when packed
450 in fuel beds. Stevens et al. (2020) have recently created a quantitative ranking of fire resistance
451 in North American conifer species across space based on plant traits, illustrating progress made
452 on scaling-up from plant functional traits to land-scale.

453 Based on these previous studies, we can thus arguably expect that changes and relationships
454 observed in our lab study could also occur in the field and that accordingly, *Q. coccifera* litter
455 flammability could increase in terms of ignitability and sustainability (burn longer) under the
456 aridity scenario predicted in the Mediterranean region. This hypothesis mainly relies on two
457 assumptions. First, under drier conditions litter water content is lowered and *Q. coccifera*
458 produces smaller leaves while maintaining litter production as recently shown (Rodriguez
459 Ramirez et al., 2017). Second, temperatures reached on soil surface during burnings of *Q.*
460 *coccifera* garrigues range between 250-400 °C (Trabaud 1979), well above the flash points of
461 alkane waxes reported in this study. Nonetheless, future research would be necessary to conduct
462 burns in plots submitted to natural and aggravated drought to test if plots from the later
463 treatment feature higher flammability as shown in this study.

464 Such hypothesized shift in litter *Q. coccifera* flammability might have limited negative
465 consequences on *Q. coccifera* shrubland vulnerability to fire since increases of fire hazard
466 would be compensated by the capacity of *Q. coccifera* to resprout after fire. Its dense
467 underground structures (e.g. woody rhizome) provide a rich nutrient reservoir (Ferran et al.,
468 2005), allowing *Q. coccifera* to be a pioneer species during vegetation regeneration in post-fire
469 succession (Arnan et al., 2007).

470 Generalizing these results to fire prone ecosystems is still speculative but this study supports
471 that the increase of climatic change-related drought could increase fuel flammability through
472 decreases of fuel moisture content (Fares et al., 2017) and concentration increases of flammable
473 leaf chemicals like waxes universally present in the plant kingdom. Such relationship is more
474 likely to occur in sclerophyllous species since they allocate an important fraction of their
475 resources to produce cuticular waxes.

476 **Acknowledgements**

477 We thank our colleagues from CEFÉ UMR 5175 (Montpellier, France) and from IMBE UMR
478 7263 (Marseille) for their contribution to the set-up of CLIMED site. We also greatly thank
479 Caroline Lecareux for assistance during chemical analyses and Sylvie Dupouyet for assistance
480 during field work. Many thanks to our colleagues from EMR team of IRSTEA (Aix-en-
481 Provence, France) for allowing us to use their equipment for flammability and leaf area
482 measurements. We are also grateful to Carmen Díez and María Luisa Cáceres from INIA for
483 their contribution to gross heat content measurements.

484 **Funding**

485 This project was partially supported by Spanish R&D project VIS4FIRE (Integrated
486 Vulnerability of Forest Systems to Wildfire: Implications on Forest Management Tools,
487 RTA2017-00042-C05-01), co-funded by the EU through the FEDER program, and was
488 possible thanks to financial contribution of the different laboratories for carrying out novel
489 studies.

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