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Increasing cuticular wax concentrations in a drier climate promote litter flammability

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Abstract

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

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

1 Introduction

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

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

The importance of terpene content in flammability of green or dead leaves in litter is however only relevant in terpene storing species. All vascular species possess waxy compounds in the upper cuticle layer (epicuticle) whose potential role in vegetation flammability could be noticeable. Epicuticular waxes cover the leaf forming a lipophilic layer which play numerous ecophysiological roles including evapotranspiration regulation, limitation of herbivore damage (e.g. anti-climb surface) and increasing UV reflection (Shepherd and Wynne Griffiths, 2006). Both, cuticle production and epicuticular wax accumulation can thus be naturally reinforced in plant species to increase tolerance to water deficit for example (Kosma et al., 2009; Xue et al., 2017). The composition of the epicuticle is a complex mixture of long-chain aliphatics, commonly named waxes, most of which can be classified as alkanes, alkanols, alkanals, alkanones, alkanoic acids and alkyl esters. Among all these compounds, alkanes are considered as the only ubiquitous waxes since they are present in the cuticle of almost all vascular plant species (Heredia and Dominguez, 2009). These compounds, which mostly range from C_{21} to C_{35}, are also of particular interest due to their influence on flammability. The flash point, the temperature at which a liquid fuel gives off sufficient vapor resulting in a flash fire when an ignition source is present, ranges from 120 to 356 °C respectively (as reported in several chemical manufacturer's Material Safety Data Sheets). Based on the relatively high flammability of alkane-waxes and the potential role of waxes to limit water losses in plants, it is important to evaluate whether aggravated drought expected under Mediterranean climate change can lead to an increased accumulation of alkane-waxes in green or litter leaves eventually favoring their flammability.

It deserves special attention to evaluate traits that influence litter flammability since this fuel is ubiquitously present in all vegetation formations, fires often start in litter and fire behavior is influenced by surface fuels and senesced leaf litter in particular (Plucinski and Anderson, 2008; Varner et al., 2015). Moreover, litter provides relatively extensive surface loads of fuels with poor moisture content in which individual leaves are easy to ignite and thus contribute to the initial fire propagation (Plucinski and Anderson, 2008). Then, well-connected surface fuels enable fire to spread easier since they influence its transmission to the upper vegetation (Belcher, 2016).

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

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

2 Materials and methods

2.1 Experimental site and design

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

Rain exclusion (recurrent aggravated drought treatment) was carried out in 10 rain plots set-up in October 2011 that received on average 30 % less rainfall compared to control plots (Fig. 1). Each rain exclusion plot presented 16 m^2 surface and was equipped with a 4 m × 4 m solid aluminum frame held 2 m above the ground, using aluminum posts at the outer circumference of the 16 m^2 plot area and fixed to the ground with reinforcing bars. Stainless steel gutters were mounted on top of the aluminum frame. A supplementary PVC gutter and a pipe mounted at the border of the frame allows for evacuation of rainwater away from the plots. The control plots consisted of 10 other plots (16 m^2, 4 m × 4 m) adjacent to the rain exclusion plots. Control plots received 100% rain since they did not present any solid frame above the ground. Leaf litter of *Q. coccifera* was collected in the 4-m^2 central zone of each plot to avoid edge effect. In all plots (rain exclusion and control plots), *Q. coccifera* was the major species with at least 80 % of *Q. coccifera* cover. Litter from the 20 plots was collected in February 2017, that is, 5.5 years after rain exclusion was applied. Only superficial and entire leaf litter was collected, corresponding to litter from the first stages of decomposition.

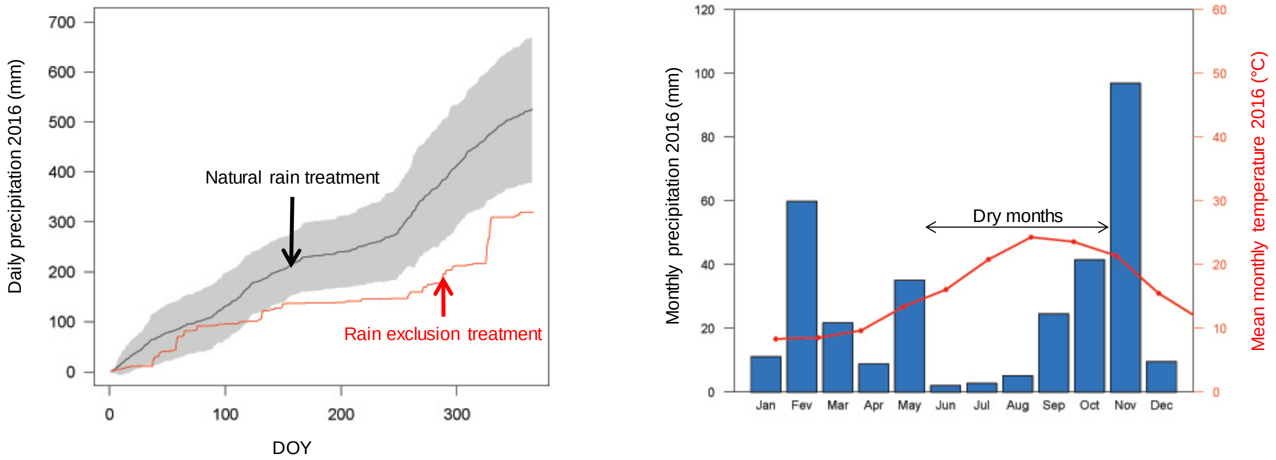

Fig. 1 Cumulative precipitation (left graph) and ombrothermic diagram (right graph) for the experimental site (CLIMED) in 2016, before litter was collected (February 2017). For cumulative precipitation, mean (line) and standard deviation (shaded area) are represented.

2.2 Litter conditioning before physico-chemical analysis

Leaf litter was partially dehydrated before any chemical or flammability analysis. This approach resulted in an average litter moisture content just below 15 % (13.2 ± 0.4 and 13.0 ± 0.5, mean ± standard error, under natural and recurrent aggravated drought respectively), since litter is extremely flammable below this threshold (Whelan, 1995; de Dios et al 2015). Litter was however not completely dehydrated to work under realistic litter moisture contents during summer months (Larchevêque et al 2005, data from CLIMED). Partial dehydration was achieved by oven-drying leaf litter at 35 °C during 4 days. Litter moisture content was calculated as follows:

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

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

2.3 Flammability measurements

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

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

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

2.4 Epicuticular alkane extraction and analysis

Epicuticular wax extraction did not aim to be exhaustive but was focused on alkane extraction alone. For this purpose, 1 g of partially dried litter was immersed into 6 ml of an organic solvent mixture formed by cyclohexane and chloroform (70:30 volume) containing a fixed amount (37.1 ng) of an internal standard (IS, undecane). This IS was not naturally present in litter leaves. Within each plot, we performed three extractions from the same litter pool (totaling 60 extractions). Extractions were performed with constant shaking for 60 s. The extract was filtered with a PTFE syringe filter (0.2 µm, 30 mm diameter). Analyses were performed using a gas chromatograph (GC, Hewlett Packard GC6890®) coupled to a mass spectrometer (MS, HP 5973N). The HP-5MS capillary column (30 m) in the GC was in constant flow mode and was connected directly to the MS. Sampled volumes (1 µl) of each alkane extract were injected through an automatic injector (ALS 7683). Helium (99.995 %) was used as carrier gas. The oven temperature was initially set at 50 °C and then it increased to 160 °C at a rate of 2 °C min^{-1}. It then remained constant for 5 min. Epicuticular alkanes were identified by comparison of the retention time and mass spectrum of detected compounds with those of the authentic reference samples (n-C_{21}-C_{40} alkane standard from Aldrich–Firmenich). Their identity was then confirmed with generated libraries of retention indexes (Adams 2007). Alkanes were quantified by considering their relative response factor (RRF) using the following formula:

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

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

2.5 Total cuticle extraction

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

2.6 Leaf area and specific leaf area measurements

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

2.7 Statistical analysis

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

3 Results

3.1. Litter physico-chemical traits under drought treatments

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

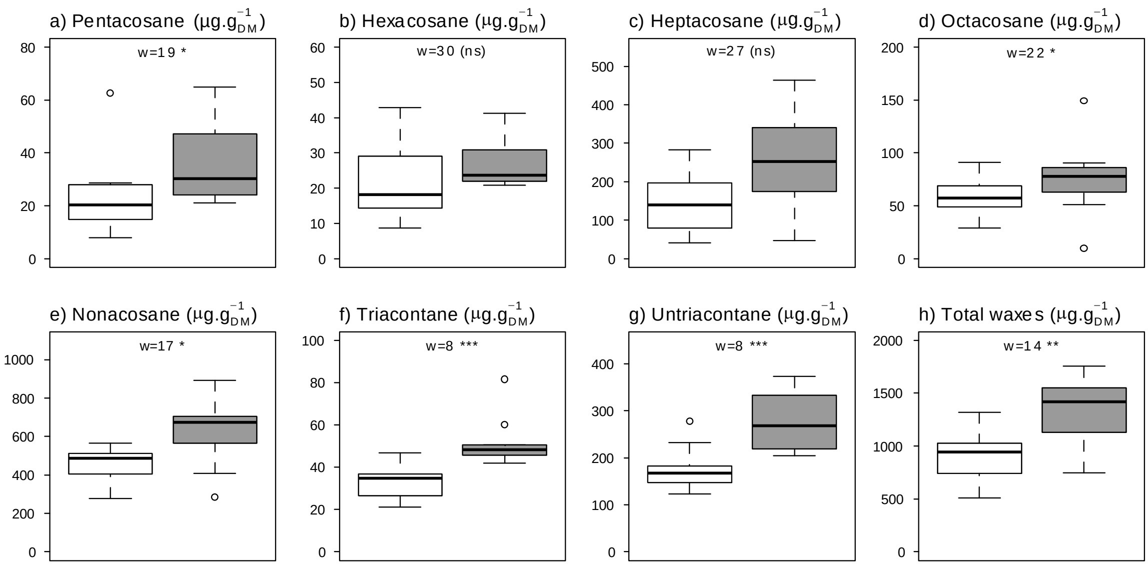

Fig. 2 Concentration (µg g_{DM}^{-1}) of n-alkanes in litter epicuticle of *Q. coccifera* under natural (white) and aggravated (grey) drought. Differences between both treatments are tested with Mann-Whitney tests (w). The central line is the median, the edges of the box extent from 1st quartile to 3rd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

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

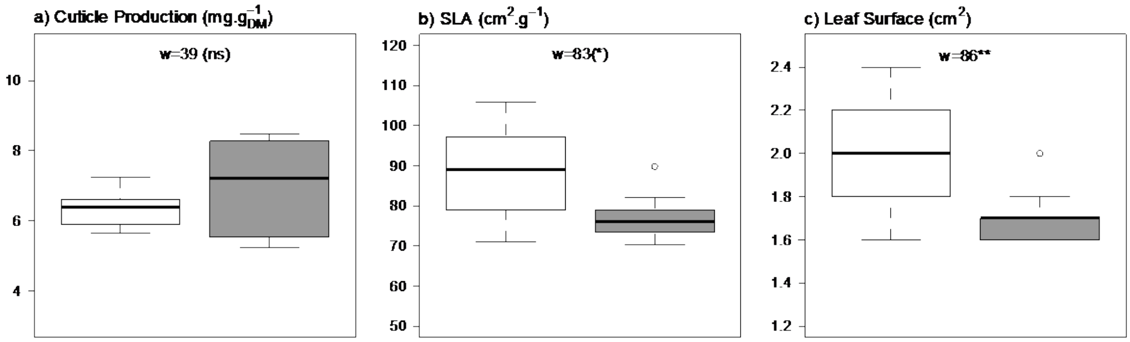

Fig. 3 Leaf litter physical traits under natural (white) and aggravated (grey) drought conditions. The central line is the median, the edges of the box extent from 1st quartile to 3rd quartile (25-75 %) and the limits of the whiskers extent to the 1.5 interquartile range. Significant differences are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

3.2. Litter flammability: variability with drought and correlations with physico-chemical traits

Litter from the aggravated drought treatment exhibited a shorter ignition delay, longer flame residence time and higher maximum flame height (Fig. 4a-c, P<0.05) compared to litter from the natural drought treatment. No effect of drought treatment was detected for gross heat of combustion (Fig. 4d).

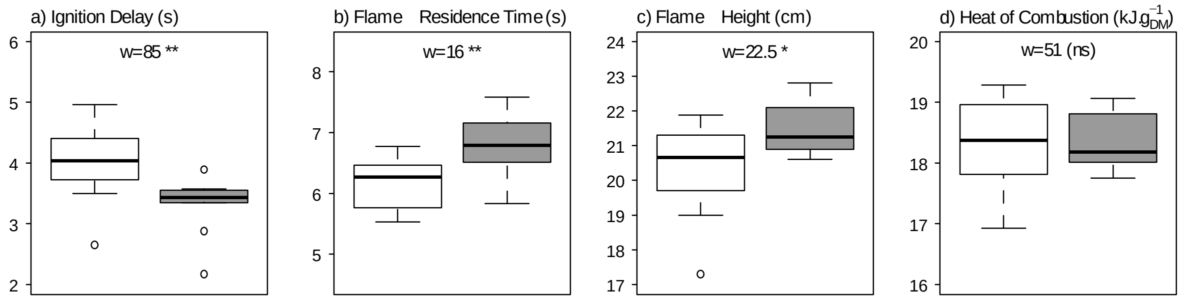

Fig. 4 Leaf litter flammability when litter originates from natural (white) and aggravated (grey) drought conditions. The central line is the median, the edges of the box extent from 1st quartile to 3rd quartile (25-75 %) and the limits of the whiskers extent to the 1.5 interquartile range. Points indicate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

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

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

Flammability parameter / Physico-chemical trait	Ignition delay (s)	Flame Residence Time (s)	Maximum flame Height (cm)	Gross heat of combustion (KJ g_{DM}^{-1})
Pentacosane ($\mu g\ g_{DM}^{-1}$)	-0.49*	0.41(*)	0.13	0.15
Hexacosane ($\mu g\ g_{DM}^{-1}$)	-0.50*	0.34	0.31	0.42(*)
Heptacosane ($\mu g.g_{DM}^{-1}$)	-0.26	0.44*	0.11	0.33
Octacosane ($\mu g\ g_{DM}^{-1}$)	-0.44(*)	0.35	0.27	0.49*
Nonacosane ($\mu g\ g_{DM}^{-1}$)	-0.49*	0.28	0.27	0.44(*)
Triacontane ($\mu g g_{DM}^{-1}$)	-0.52*	0.39(*)	0.50*	0.40(*)
Untriacontane ($\mu g\ g_{DM}^{-1}$)	-0.39(*)	0.28	0.31	0.33
Total alkane production ($\mu g\ g_{DM}^{-1}$)	-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

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

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

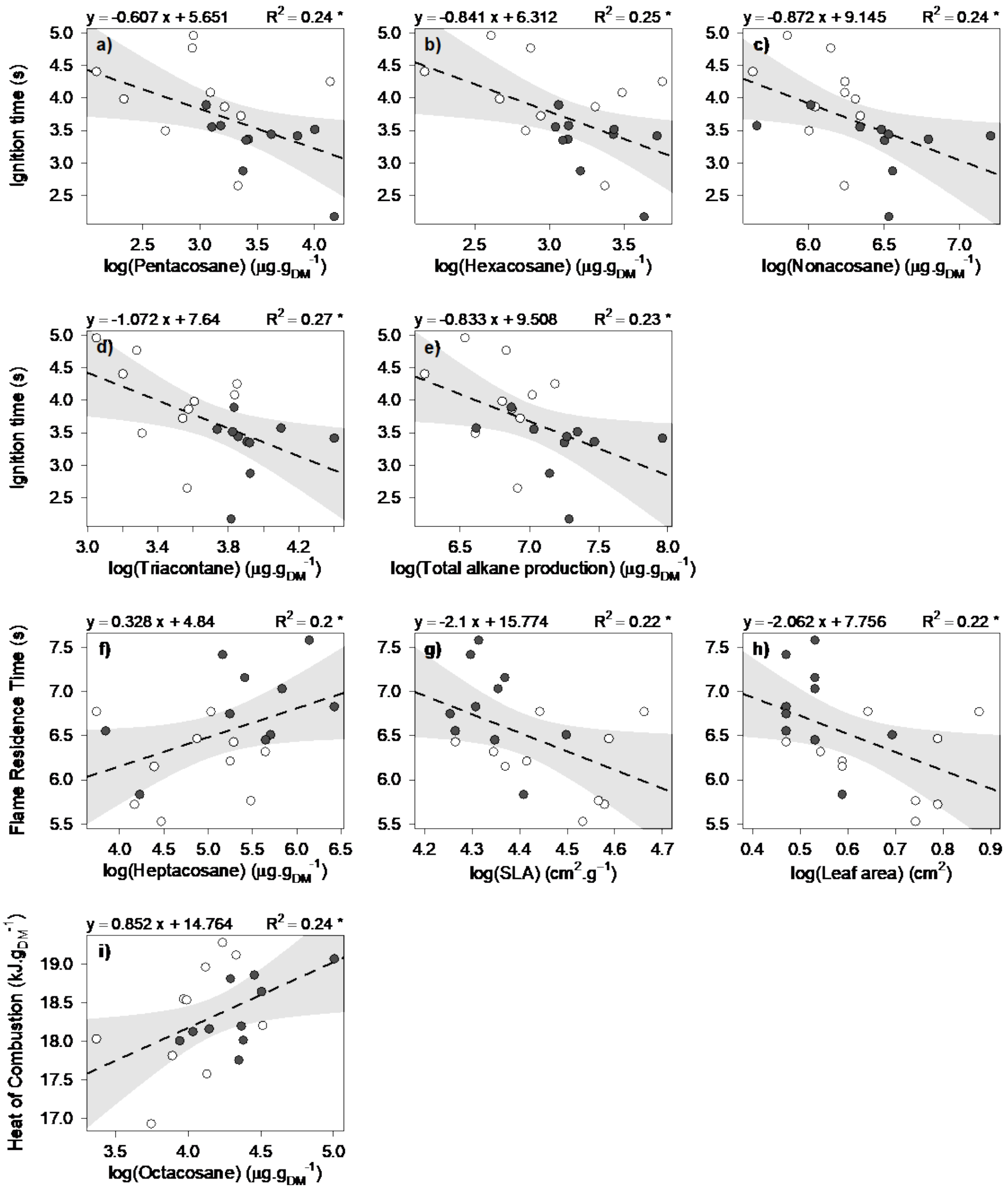

Fig. 5 Pearson correlations between physico-chemical traits (x axis) and flammability parameters using log-transformed data (y axis). Black and white points refer to natural and aggravated drought treatments respectively. Points represent the average ± SE values (n=10). Shaded grey represents the prediction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

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

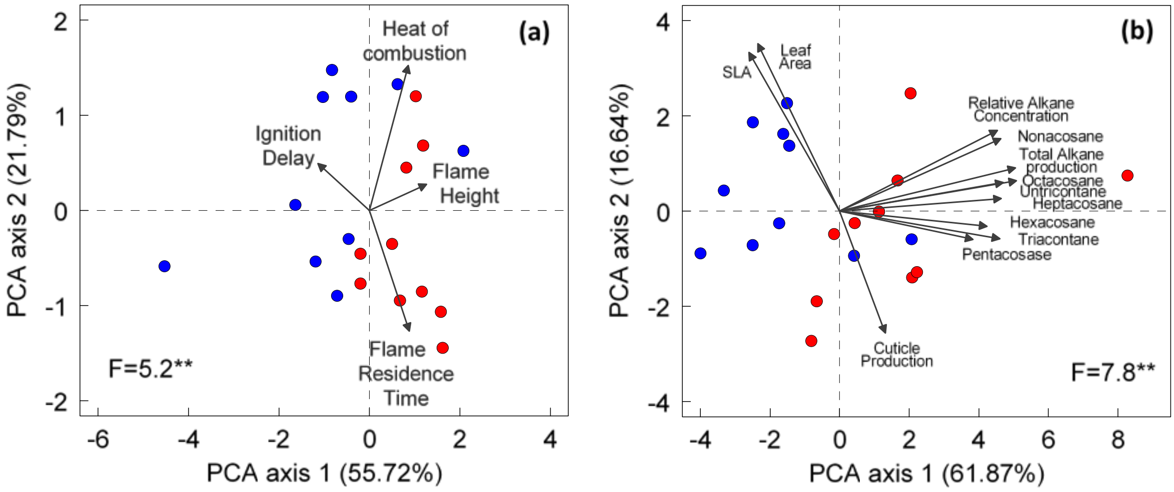

Fig. 6. Principal Component Analysis showing differences between natural (●) and aggravated drought (●) in terms of **(a)** leaf flammability metrics (maximal flame height and ignition delay explain axis 1 and gross heat of combustion and flame residence explain axis 2) and, **(b)** physico-chemical variables (most explanatory variables are alkanes from C_{27} to C_{31} and total alkane content in axis 1 and SLA, leaf area and cuticle production in axis 2). Percentages refer to variability explained by each axis. PERMANOVA tests (F) followed by ** denote the significance of the differences between the treatments (**: 0.01< P <0.001). The axes scores of each variable are provided in Table S3.

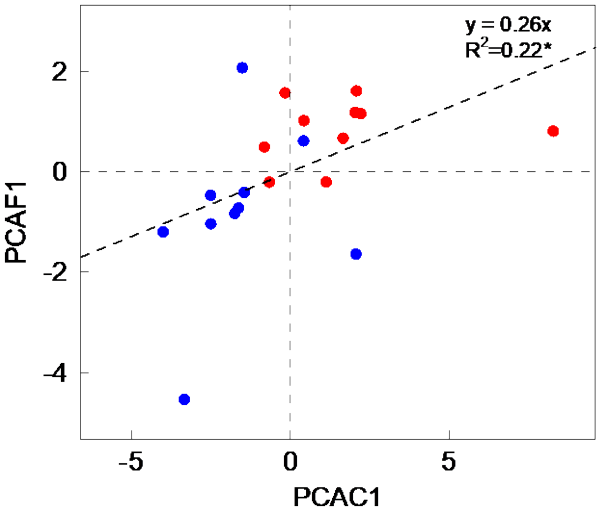

Fig. 7. Correlation between the axis scores resulting from the physico-chemical litter traits based-PCA (x axis) (obtained in Fig. 6a) and axis scores resulting from the leaf flammability based-PCA (y axis) (obtained in Fig. 6b).

4 Discussion

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

4.1 Chemical and physical trait influence on leaf litter flammability

Most alkanes in litter cuticle of *Q. coccifera* presented an odd number of carbon atoms, as commonly shown in other species (Diefendorf et al., 2015). Nonacosane (C_{29}) was the major compound in accordance with the epicuticular alkane composition found in *Q. ilex*, *Q. suber* and *Q. calliprinos* (Palestine oak) (Martins et al., 1999; Brosh et al., 2003; Norstrom et al., 2017). Wax concentration and coverage in leaves is extremely variable among species, from 0.4 (minimum wax coverage) to 160 µg cm^{-2} (maximum wax coverage) (Deininger, 2016). In view of our results, alkane concentration in leaf litter of *Q. coccifera* (1189.4 µg g_{DM}^{-1} or 14.8 µg cm^{-2}) can thereby be considered a major alkane cover, in the range of those found in cuticles of green leaves of other Mediterranean sclerophyllous species like *Q. suber* (18 µg cm^{-2}), *Q. ilex* (7 µg cm^{-2}) (Martins et al., 1999) and *O. europaea* (*8.5* µg cm^{-2}) (Huang et al., 2017), which is well above the alkane concentration in conifer species (from dizaines to less than 900 µg g_{DM}^{-1} (Diefendorf et al., 2015).

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

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

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

4.2. Understanding litter trait changes according to water deficit

The high epicuticular alkane concentration in leaf litter under aggravated drought was probably due to the combination of both, limited microbial activity under limited water conditions which slows down the litter decomposing process (Ormeño et al., 2006; Santonja et al., 2015) and plant history, although none of these points were checked in this study. When litter fall occurs, some green leaf traits must be reflected in litter at least during the first stages of decomposition. Epicuticular wax coverage in green leaves fulfills major biological functions in living plants including water conservation (by preventing non-stomatal water loss), nutrient conservation and limiting abiotic and biotic stress-related damage (Shepherd and Wynne Griffiths, 2006; Barthlott et al., 2017). Thus, under water scarcity, wax accumulation in the leaf cuticle often increases (Bacelar et al., 2012). Under aggravated and recurrent drought, *Q. coccifera* litter also exhibited smaller leaves resulting in smaller SLA and so potentially thicker and/or denser leaves. Such responses under aggravated drought (high leaf wax cover, small leaf area, low SLA) are characteristic of sclerophyllous species (Gratani and Varone, 2006) and probably allow *Q. coccifera* to maximize its fitness under scarce water conditions in fire-prone ecosystems. Increasing wax load and reducing leaf area would limit evapotranspiration during long-term drought while implying a higher fire hazard with minimal stand disturbance since post-fire resprouting is the main recolonisation mechanism of *Q. coccifera*. This species possesses the ability to regrow after burning by shoots from stocks and suckers from the roots (Konstantinidisa et al., 2005).

4.3. Potential management and ecological implications of these findings

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

It is worth noting that epiradiator tests do not pretend to mirror what occurs in the field. In fact, each measurement scale (leaf-scale, fuel bed scale, field burning) provides different information. Epiradiator tests do not integrate fuel structure but are an adequate approach to assess the importance of different leaf traits on some flammability metrics and the best choice for this study since each experimental plot possesses 16 m^2 surface and only litter from the central 4 m^2 was collected. Thus, important amounts of litter cannot be collected within each plot impeding the use of leaf litter beds to study flammability. Although burnings performed in the field provide better estimates of fire behavior in a given ecosystem, explaining the factors involved in fire behavior is a complex task due to the influence of multiple biotic factors under natural conditions. For example, in the study of Trabaud (1979) based on burnings of *Q. coccifera* in different plots in a garrigue in Southern France, the authors noted that *Q. coccifera* fire behavior and flammability was influenced by the high water content of grasses (co-existing with *Q. coccifera)*, which was by far larger compared to woody species.

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

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

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

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

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