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Fragmentation reduces severe drought impacts on tree functioning in holm oak forests

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ABSTRACT

Fragmentation and increased summer drought are two main threats to Mediterranean forests. Forest fragmentation has many negative impacts on forests but could attenuate water stress on Mediterranean species by reducing intraspecific competition or improving soil properties at forest edges. However, little is known about the combined effects of drought and fragmentation on tree functioning. We evaluated the effect of forest fragmentation on tree functioning under severe drought periods in continental holm oak forests (Quercus ilex). We monitored the functional response of focal trees before and during summer drought in two regions of the Iberian Peninsula with contrasting climates. Forest interiors, edges and small forest fragments were compared. Predawn leaf water potential (Ψ_{pd}), leaf stomatal conductance to water vapor (g_s), maximum leaf photochemical efficiency (F_v/F_m) , ground (F_o) and maximum (F_m) fluorescence in dark adapted, fully-developed leaves were assessed in the field. Trees located at forest interiors and edges showed strong stomatal closure and clear symptoms of photochemical inhibition, revealing that the trees were operating at predawn water potential beyond tolerance thresholds. In contrast, trees located in small forest fragments were able to maintain high levels of functionality for all ecophysiological parameters during the drought. These differences in tree functioning among fragments were more noticiable in the driest region. Our results prove that forest fragmentation attenuates drought impacts on tree functioning, and that these positive effects become more important during extremely dry periods. These novel findings are essential for realistic predictions of the functionality of fragmented forests under increasing and more severe droughts.

1. Introduction

Increased aridity due to climate change and habitat fragmentation are the main global change drivers affecting forest ecosystems in the Mediterranean region (Matesanz and Valladares, 2014; Valladares et al., 2014a). Water scarcity, especially during summer, is expected to increase due to more frequent and intense droughts (IPCC, 2014). Such extreme drought events limit water use and plant growth (Flexas et al., 2014; Forner et al., 2014). Then, these physiological processes, resources uptake and secondary growth, can be decoupled from each other which could decrease tree recovery ability and eventually lead to tree decline (Barbeta et al., 2015; Bréda et al., 2006; Lloret et al., 2004). Several dieback episodes in drought-prone areas have raised concern about the ability of Mediterranean trees to cope with severe water deficit despite their adaptations to drought (Gentilesca et al., 2017). Improving our understanding of the physiological mechanisms underlying drought-induced tree decline will help both to improve predictions and to develop early-warning systems (Colangelo et al., 2017).

Concomitantly to drier conditions, land use changes in the Mediterranean basin have entailed habitat fragmentation (Valladares

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et al., 2014b), which is expected to increase over the coming decades (Haddad et al., 2015). Key processes such as seed dispersal, seedling recruitment, plant-animal interactions (Aparicio et al., 2012; Morán-López et al., 2015; Santos and Tellería, 1997) and genetic diversity (Ortego et al., 2010, 2014) can suffer detrimental effects from fragmentation. These effects are caused by the combination of habitat reduction, increased isolation and changes in the physical environment (Valladares et al., 2014a). Most studies have addressed the separate effect of fragmentation and drought on plants obviating their temporal parallelism and inter-dependence (Parmesan et al., 2013), so there is scant information on the combined effects of these two factors. Some studies suggest that fragmentation exacerbates the negative impacts of increased aridity due to higher exposure to radiation and more intense evapotranspiration (Leimu et al., 2010; Matesanz and Valladares, 2014), increasing the vulnerability of forest species to drought (Opdam and Wascher, 2004; Travis, 2003). Conversely, others point out that fragmentation can attenuate water stress due to reduced tree-to-tree competition at forest edges (Morán-López et al., 2016) or to increased soil moisture due to matrix loadings (Flores-Rentería et al., 2018b). Surprisingly, previous studies in fragmented forests have overlooked the functional status of individual plants despite its relevance for coping with severe drought periods (Flexas et al., 2014). Little is known about the joint effect of fragmentation and drought on tree functioning (but see Leimu et al., 2010; García-Valdés et al., 2015). Therefore, it is essential to elucidate if the observed reduction in water stress by fragmentation leads to an actual improvement of tree functioning during drought.

Holm oak (Quercus ilex) is a widely distributed species in Mediterranean ecosystems where it has a prominent economic and ecological role. In several regions of Spain, holm oak forests have been fragmented since the 1950s, reducing the average size of forests to a quarter of the area and turning the forests into croplands (Santos and Tellería, 1998). This fragmentation process was very fast, so that in only 50 years (1946-1996) some forest areas (e.g. Burgos) were reduced by 55.6 % (Santos and Tellería, 1998). Q. ilex is considered a drought-tolerant species, and, as such, is able to maintain carbon fixation under high levels of water stress (Chaves et al., 2002; Corcuera et al., 2004; Forner et al., 2018; Quero et al., 2011), tolerating low leaf and xylem water potentials and high leaf tissue desiccation (Forner et al., 2018). However, this may lead to higher risks of functionality loss of its hydraulic system (Rodríguez-Calcerrada et al., 2017; Urli et al., 2013). Such loss of tree functionality was reported as the main cause of tree decline in the western Mediterranean region (Gentilesca et al., 2017), which has led to high defoliation rates and dieback episodes (Camarero et al., 2004; Hereş et al., 2018; Peñuelas et al., 2001). Strikingly, in previous work we found that holm oaks located in fragmented areas, did not suffer acute water shortage thanks to reduced tree-to-tree competition (Morán-López et al., 2016). Nonetheless, it is not clear whether the observed reduction of water stress can improve tree condition during drought periods, which may result in a lower vulnerability in the medium-term.

We hypothesized that fragmentation attenuates the negative impacts of drought on tree functioning as observed with tree water stress. We assessed drought effects on holm oaks from two climatically distinct regions (northern, mesic vs southern, xeric), in woodlands with different levels of fragmentation and in years with contrasting drought intensity. In addition, we expected that the inter-play between fragmentation and drought on tree functioning would be reinforced during drier environmental conditions, i.e. in the southern region and during the driest year.

2. Materials and methods

2.1. Study regions

The study was carried out in two holm oak (Quercus ilex subsp.

ballota) fragmented forests of the Iberian Peninsula, which represent contrasting climatic conditions. In both regions, the forests were fragmented as result of the agriculture's expansion rendering a variety of patch sizes. The fragmentation process was very fast and started more than 60 years ago in both forests (Santos and Tellería, 1998), so adult trees have approximately the same age. The surrounding matrix were wheat and legume croplands, with scattered grape crops. This agricultural matrix was not irrigated in any of the studied regions. The studied fragments were selected near Lerma (42°5'N, 3°45'W; 930 m a.s.l.) in the northern region, and near Quintanar de la Orden (39°35'N, 3°02'W; 870 m a.s.l.) in the southern region of the Iberian Peninsula. In both regions, the dominant forest tree species is holm oak, with an understory composed by several shrub species (see Santos and Tellería, 1998; Díaz et al., 1999; Morán-López et al., 2016 for further details in the species' composition). In both regions, the fragments had similar vegetation with similar spatial distribution, and were separated by at least 50 m and as much as 11 km. The dominant soils are Cambisols (WRB, 2007) with 11 % sand, 42 % silt and 47 % clay for the northern region, and 17 % sand, 39 % silt and 44 % clay for the southern region (Flores-Rentería et al., 2015). In the northern region, annual precipitation is 567 mm and annual mean temperature is 11 °C, and in the southern region, 342 mm and 15.8 °C, respectively (from AEMET data, mean 1982-2013: Villafría meteorological station at 39 km away from Lerma; 891 m a.s.l.; from Toledo meteorological station at 89 km away from Quintanar de la Orden; 515 m a.s.l.). Following Morán-López et al. (2016) who found that water shortage during summer was on average 68 % more severe in the southern than in the northern region, we expected the summer droughts to be more intense in the southern than in the northern region.

2.2. Experimental design

We defined three ecological situations with regard to fragmentation in both regions: (1) forest interiors and (2) edges in large forest fragments, and (3) small fragments. In each study region, we selected three large forest fragments comprising continuity of forest vegetation (>100 ha), and 10–11 forest small fragments (mean of 0.4 ha) surrounded mainly by agricultural fields. Most of the small fragments were no further away than 8–10 km from large forest fragments. In large fragments, we defined forest interiors as at least 60 m from the cultivated border (García et al., 1998). Forest edges were selected along straight borders to avoid influences of border geometry (Fernández et al., 2002). In each fragmentation level and site, we randomly selected between 26 and 28 trees equally distributed in the different replicates of fragmentation levels (total sample size = 163).

In general, fragmentation levels differed in soil characteristics, structural properties of trees and tree-to tree competition (Table 1). In the northern region, soils from the forest interiors showed lower organic matter and pH (see Flores-Rentería et al., 2015 for methods' information). In the southern region, patterns were similar, but differences were not significant. In all cases, slope, soil stone content and aggregates did not differ across fragments. In addition, previous studies carried out in the same fragments show significant effects of forest fragmentation on other soil characteristics (Flores-Rentería et al., 2015, 2018a,b, 2016). In general, in small fragments, trees are taller, have higher canopy projection and a higher basal area. Such differences are more acute in the southern region (Table 1). These differences should not be due to differences in tree age since the beginning of the fragmentation process dates back to the 1950s in both regions (Santos and Tellería, 1998). The reason of these differences may be that trees from small fragments: 1) have had better growing conditions due to the incoming nutrients from the surrounding matrix (Flores-Rentería et al., 2016); 2) are surrounded by the most fertile soils from the primitive forest (Santos and Tellería, 1998); 3) have had different exploitation situations while forest interiors have been used systematically for firewood, grazing or carbonating (Santos and Tellería, 1998). Finally,

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Table 1

Mean \pm SE of height (m), basal area (m²) and canopy projection (m²) of the selected trees, intraspecific competition (proportion) and soil organic matter (%), soil pH and soil aggregates (%) measured in each fragmentation level (Interior, Edge and Small) and in the northern and southern region. Different letters indicate results of Tuckey post hoc analysis between fragmentation levels in each region (P < 0.05). n.s. indicates non-statistically significant differences.

	Northern Region			Southern Region			
	Interior	Edge	Small	Interior	Edge	Small	
Height	4.08 ± 0.21 b	4.88 ± 0.31 ab	5.67 ± 0.24 a	4.18 ± 0.26 b	4.22 ± 0.29 b	6.27 ± 0.31 a	
Basal area	0.06 ± 0.008 n.s	0.083 ± 0.014 n.s	0.114 ± 0.032 n.s	0.063 ± 0.011 ab	$0.031 \pm 0.005 \text{ b}$	0.099 ± 0.014 a	
Canopy projection	15.15 ± 1.89 b	21.04 ± 2.70 ab	30.46 ± 5.28 a	31.33 ± 7.90 n.s	17.93 ± 2.68 n.s	36.30 ± 4.08 n.s	
Intraspecific competition	$0.65 \pm 0.02 a$	$0.52 \pm 0.02 \text{ b}$	$0.31 \pm 0.08 c$	0.46 ± 0.04 a	$0.36 \pm 0.03 \text{ b}$	$0.27 \pm 0.14 \text{ b}$	
Soil organic matter (%)	6.59 ± 1.16 b	17.64 ± 2.01 a	19.84 ± 2.39 a	8.42 ± 0.46 n.s.	12.19 ± 0.71 n.s.	14.09 ± 1.75 n.s.	
Soil pH	6.75 ± 0.37 b	7.69 ± 0.07 a	7.32 ± 0.11 ab	7.85 ± 0.06 n.s.	7.88 ± 0.04 n.s.	7.87 ± 0.03 n.s.	
Soil aggregates (%)	50.84 ± 3.43 n.s.	65.46 ± 2.79 n.s.	57.14 ± 3.98 n.s.	46.86 ± 2.07 n.s.	48.81 ± 7.34 n.s.	49.07 \pm 2.87 n.s.	

trees located in small forest fragments show values of intraspecific competition between a 41 and 52 % lower than in forest interiors (southern and northern region, respectively, Table 1). Intraspecific competition was calculated as the oak cover proportion within a radius of 20 m around the focal tree (see Morán-López et al., 2016 for method's information).

In order to evaluate the impact of summer drought on the ecophysiological response of the selected trees, each year we sampled in two periods (before and during drought): early summer (ESu, second fortnight of June) and late summer (LSu, second fortnight of August). The study was carried out in two years that highly contrasted in the intensity of drought (2012 and 2013) as described in Morán-López et al. (2016). The nearest meteorological stations with complete data for the study years (Sourthern region: Ocaña, at 57 km from Quintanar de la Orden, 733 m a.s.l.; Northern region: Villamayor de los Montes, at 13 km from Lerma, 882 m a.s.l.) were used to evaluate the magnitude of summer drought. The summer drought of 2012 was extreme compared with the long-term mean, so we consider 2012 a dry year and 2013 a wet year.

2.3. Data collection

In both study periods and years, water stress was assessed from predawn leaf water potential (Ψ_{pd} , MPa), measured in two twigs per tree with a Scholander pressure chamber (Scholander et al., 1965). We followed a randomized factorial design to avoid the effect of daily meteorological conditions in ecophysiological measurements, so each day we measured approximately the same number of trees per forest fragmentation level (15 trees). Excised twigs were collected at predawn, enclosed in sealable plastic bags with air saturated of humidity and CO₂ and kept refrigerated and in darkness until measurements were performed (Pérez-Harguindeguy et al., 2013).

To assess overall tree functioning, we measured several functional variables. Stomatal conductance (g_s , mmol s^{-1} m⁻²) was measured as a proxy of carbon fixation in 4-6 leaves per tree with a leaf porometer SC-I (Decagon, Pullman, USA) at mid-morning, when species maximal conductance is expected (10:00-12:00 h). Predawn and midday ground (F_{opd} and F_{omd} , respectively; unitless) and maximum (F_{mpd} and F_{mmd} , respectively; unitless) fluorescence were measured in 4-6 dark-adapted leaves per tree with a portable pulse-modulated fluorometer FMS2 (Hansatech, Norfolk, UK) to evaluate the functioning of the photosystem II (Briantais et al., 1996). F_{0} indicates the minimal fluorescence emission at the full open centers without photochemical quenching. The increase in F_o may indicate direct disease in PSII centers or the inability to transfer excitation energy from antenna to active centers (Bolhar-Nordenkampf et al., 1989). Measurements were done in full sun leaves orientated to South, close to those used to measure g_s, and within easy reach. The distances among trees were long enough to avoid shading and to allow sampling of comparable sunlit leaves. Predawn and midday maximum photochemical efficiency of photosystem II (F_v /

 $F_{\rm m} = (F_{\rm m} - F_{\rm o})/F_{\rm m}$) was estimated from $F_{\rm o}$ and $F_{\rm m}$ ($F_{\rm v}/F_{\rm mpd}$ and $F_{\rm v}/F_{\rm mpd}$ $F_{\rm mmd}$, respectively; unitless) to evaluate photosynthetic functioning (Maxwell and Johnson, 2000). A decrease in predawn F_v/F_m indicates a chronic photoinhibition and allows evaluation of the reversibility of negative impacts from drought on the leaf photochemical efficiency (Werner et al., 2002). Midday measurements were done from 13:30 to 15:30. Differences between predawn and midday measurements were calculated for the three variables $(\Delta F_v/F_m, \Delta F_o \text{ and } \Delta F_m)$ to test the effect of daily adjustment in the photochemistry from dawn to midday. Current- and previous-year leaves (CYL and PYL, respectively) may have differences in biochemical composition and photoprotective characteristics due to their different ages (Camarero et al., 2012; Munné-Bosch, 2007), or due to being exposed to previous droughts that determines their vulnerability to a new drought. To account for such variability, we measured stomatal conductance and fluorescence in both kinds of leaves (2-3 leaves per tree and type).

To evaluate drought-induced differences in structural functional traits, we measured leaf dry matter content (LDMC, mg g⁻¹) and specific leaf area (SLA, m² kg⁻¹) (Garnier et al., 2001) in PYL in LSu and ESu, respectively. LDMC and SLA are related with sclerophylly, nutrient limitation and photosynthetic rate (Pérez-Harguindeguy et al., 2013). LDMC was calculated in LSu and in both years from three PYL per tree dividing each leaf's oven-dry mass by its water-saturated fresh mass. Leaves were fully rehydrated in darkness for 12 h. SLA was estimated in ESu and in both years from three fresh PYL per tree scanned without the petiole and oven-dried at 65 °C for 48 h. Leaf area was estimated with the software ImageJ from scanned leaves (Rasband, 2009). All leaves and twigs were sampled on the south side of the tree crowns. See Table C1 for a summary of the variables measured and their interpretation.

2.4. Statistical analysis

For each focal tree, and on year, period, region and fragmentation level bases, we calculated mean values of all variables differentiating between current- and previous-year leaves (CYL and PYL, respectively).

Linear mixed-effects models (LMMs) were used to assess the effect of year, period, region, forest fragmentation level and their interaction (fixed factors) on water stress endured by trees (Ψ_{pd}), ecophysiological (g_s , F_v/F_{mpd} , F_v/F_{mmd}) and leaf structural traits (LDMC and SLA) (Zuur et al., 2007). To account for spatial and temporal autocorrelation, focal tree nested in fragment identity was introduced as a random factor. Models were fitted based on a restricted maximum likelihood method (*nlme* package) (Pinheiro et al., 2007). Analyses were performed separately for PYL and CYL. In addition, to assess the effects of leaf type on g_s , F_v/F_{mpd} and F_v/F_{mmd} , we performed nonparametric Kruskal-Wallis tests for each year, period, region and forest fragmentation level combination.

To evaluate the effects of fragmentation and drought on overall tree functioning, we conducted Spearman's rank correlation analysis among all ecophysiological variables (Ψ_{pd} , g_s , F_v/F_{mpd} , F_v/F_{mmd} , $\Delta F_v/F_m$, F_{opd} ,

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 F_{omd} , ΔF_{o} , F_{mpd} , F_{mmd} , ΔF_{m}). Higher correlations among variables imply an overall response of tree performance. We divided data by regions, fragmentation level and types of leaves to assess if correlation strength changed accordingly. In addition, we assessed if acute water shortage could lead to low carbon fixation rates and leaf photochemical damage. For this purpose, we used non-linear and linear models between Ψ_{pd} and g_s , F_v/F_{mpd} , F_v/F_{mmd} , $\Delta F_v/F_m$ and F_{opd} as dependent variable considering means per fragmentation level and region. Differences between the slopes of the regressions were determined by the function lstrends (package *lsmeans*). In order to analyze the performance of leaf photochemistry of young and old holm oaks leaves, we used linear models between F_{opd} and F_{omd} . A departure of the slope from the 1:1 line can be interpreted as higher degree of photoinhibition. Finally, we evaluated if recurrent water shortage could be reflected in leaves structural changes by correlating Ψ_{pd} to LDMC and SLA in PYL All analyses were carried out using R statistical software (version R3.3.1; R Development Core Team, 2016, Vienna). See Table C2 for a summary of data analyses.

3. Results

3.1. Fragmentation effects on water stress, tree functioning, and structural traits

Water stress, measured as leaf water potential, differed between regions, years, periods and forest fragmentation levels (Table 2). In both regions, the lowest water potentials were detected in the dry year and in late summer (LSu) (Fig. 1). In general, forest fragmentation attenuated water stress, especially during drier conditions (southern region, dry year and late summer). Only when conditions were mild (northern region, wet year and early summer), fragmentation had no effect on tree water status (Fig. 1). In both regions, in LSu of the dry year, forest interiors and edges showed Ψ_{pd} below -3 MPa (northern: -3.19 ± 0.09 MPa and -3.24 ± 0.13 MPa; southern: -4.26 ± 0.12 MPa and -4.04 ± 0.15 MPa, respectively), while trees located in small fragments suffered moderate water stress (northern: -2.48 ± 0.14 MPa, southern: -2.84 ± 0.12 MPa). As expected, these patterns were more noticiable in the southern region, where differences in Ψ_{pd} between forest interiors and small fragments reached 33 % (compared to 22 % in the northern region).

Regarding plant performance, stomatal conductance (g_s) differed between years and periods (Table 2). Forest fragmentation only affected

 g_s when interactions with year and period were considered. In general, the lowest g_s were measured in LSu and in the dry year for all forest fragmentation levels (Figs. 1 and 2). Only when drought was more intense, we observed higher g_s in oaks located in small forest fragments. This is in LSu of the dry year in the northern region and in LSu of both years in the southern region. Positive effects of fragmentation in g_s were stronger in the southern region and in the dry year, where in LSu g_s was on average 51 % lower in forest interiors than in small fragments (vs 43 % in the northern region) (Figs. 1 and 2). Previous-year leaves (PYL) showed similar trends to current-year leaves, but fragmentation effects on g_s were weaker (Table B1, Figs. 1 and 2).

In general, the effect of forest fragmentation on photochemical efficiency of photosystem II in dark adapted leaves (F_y/F_m) depended on its interaction with the year, period and region (Table 2). In both years and regions, trees located in small forest fragments were able to maintain the highest F_v/F_m during drought (Figs. 1 and 2). In contrast, summer drought exerted a strong negative impact in trees located at forest interiors (Figs. 1 and 2, Table 2). In fact, the lowest values of F_v / $F_{\rm m}$ as well as the greatest differences between predawn and midday values $(\Delta F_v/F_m)$, were observed in trees from forest interiors of the southern region, and during LSu of the dry year (Figs. 1f,h; 2 d,f). In addition, they suffered stronger effects of summer drought on the reduction of F_v/F_{mpd} and F_v/F_{mmd} . This pattern was consistent across years and regions, especially for PYL. This general trend of reduced photochemical efficiency in forest interior was similar between CYL and PYL (Figs. 1 and 2, Table B1). Predawn and midday ground fluorescence ($F_{\rm opd}$ and $F_{\rm omd},$ respectively) and maximum fluorescence ($F_{\rm mpd}$ and $F_{\rm mmd}$, respectively) decreased throughout summer (LSu) (Fig. B1,B2). After summer drought, trees located in small fragments showed the lowest values (F_{o}) and the highest values (F_{m}), especially in the southern region (Fig. B1,B2). The relationship between F_{opd} and F_{omd} was stronger in LSu and in PYL (Fig. B3), i.e. under higher water stress conditions PYL showed higher susceptibility to photoinhibition between dawn and midday than CYL. Differences in this relationship between ESu and LSu were only significant in PYL (P = 0.0378) which corroborates a higher effect of summer water stress in PYL than in CYL.

The case of leaves structural traits, leaf dry matter content (LDMC) and specific leaf area (SLA) differed between years, regions and forest fragmentation levels (Table 2). Nonetheless, we did not detect a strong synergic effect between fragmentation and drought in any of the regions. Differences among levels of forest fragmentation in LDMC and

Table 2

Summary statistics for the linear mixed models (*F*) testing the effect of year (Ye), period (Pe), fragmentation level (Frag), region (Reg) and their interaction on the predawn leaf water potential (Ψ_{pd}), stomatal conductance (g_s), predawn (F_v/F_{mpd}) and midday (F_v/F_{mmd}) maximum photochemical efficiency of photosystem II, leaf dry matter content (LDMC) and specific leaf area (SLA). Statistically significant differences are indicated with asterisk (* $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$). n.s. indicates non-statistically significant differences. n.a. indicates information not available. Non-significant interactions removed from the statistical model are not shown.

		Ψ_{pd}	g _s		$F_{\rm v}/F_{\rm mpd}$		F _v /F _{mmd}		LDMC	SLA
			PYL	CYL	PYL	CYL	PYL	CYL		
Intercept	F	1859.00***	5518.30***	2916.87***	22075.58***	583455.50***	77460.57***	96586.77***	63663.34***	18298.02***
Ye	F	952.42***	129.87***	21.27***	33.57***	0.00 ^{n.s.}	118.77***	3.62 ^{n.s}	44.74***	200.59***
Ре	F	1636.06***	158.83***	130.84***	35.85***	83.00***	211.94***	1.79 ^{n.s}	n.a.	n.a.
Reg	F	25.68***	0.02 ^{n.s.}	0.37 ^{n.s.}	7.99**	41.40***	1.20 ^{n.s.}	0.55 ^{n.s.}	7.43*	7.01***
Frag	F	20.00***	1.68 ^{n.s.}	3.09*	25.59***	21.40***	18.75***	25.10***	4.88**	9.99**
Ye x Pe	F	128.86***	2.53 ^{n.s.}	15.44***	5.05*	30.50***	85.95***	129.94***	n.a.	n.a.
Ye x Reg	F	0.74 ^{n.s.}	67.80***	53.64***	77.01***	160.90***	40.96***	119.27***	46.05***	
Ye x Frag	F	15.63***	14.95***	30.43***	6.54**	11.90***	13.91***	9.25***		3.21*
Pe x Reg	F	0.17 ^{n.s.}	0.06 ^{n.s.}		0.08 ^{n.s.}	37.40***	0.08 ^{n.s.}	75.52***	n.a.	n.a.
Pe x Frag	F	18.74***	13.57***	18.16***	27.54***	14.20***	21.76***	11.62***	n.a.	n.a.
Reg x Frag	F	2.84 ^{n.s.}	0.34 ^{n.s}		2.08 ^{n.s.}	4.40*	4.68*	3.62*		3.28*
Ye x Pe x Reg	F	36.08***	13.57**		5.31*			4.96*	n.a.	n.a.
Ye x Pe x Frag	F	6.58**	1.67 ^{n.s}		2.53 ^{n.s.}	4.10*	10.38***	4.43*	n.a.	n.a.
Ye x Reg x Frag	F	3.61*	0.03 ^{n.s}		2.76 ^{n.s.}	3.00*	3.32*	5.11**		
Pe x Reg x Frag	F		0.01 ^{n.s}		3.57*		3.67**	17.27***	n.a.	n.a.
Ye x Pe x Reg x Frag	F		3.85*		7.02**		6.10**		n.a.	n.a.



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Fig. 1. In previous-year leaves (PYL), measurements of predawn leaf water potential (Ψ_{pd}, MPa) (a,b), stomatal conductance (g_s, f_s) mmol $s^{-1} m^{-2}$) (c,d) and predawn and midday maximum photochemical efficiency of photosystem II $(F_v/F_{mpd}$ and F_v/F_{mpd} respectively; unitless) (e,f and g,h respectively). Bars correspond to measurements (mean ± SE) performed in different fragmentation levels (Interior, Edge and Small), in early (ESu) and late (LSu) summer of both study years (dry and wet). Left and right panels correspond to the northern and southern region, respectively. For each year and period different letters indicate statistically significant differences between fragmentation levels at P < 0.05.

SLA were not clear in the northern region (Fig. B4). In the southern region, trees of small forest fragments had significantly lower LDMC and higher SLA. (see Appendix A for more information).

3.2. Effects of water stress on tree functioning and structural traits

Stronger correlations were found between ecophysiological variables and the water stress (Ψ_{pd}) in the southern region than in the northern region, especially in forest interiors, which indicated an overall higher ecophysiological stress in those than in small forest fragments (Fig. B5, B6).

Regarding the effects of water stress on tree functioning, a significant correlation was found between g_s and Ψ_{pd} (Figs. B5, B6). The mixed models performed for LSu showed a dependence of g_s on Ψ_{pd} (P < 0.0001) and on its interaction with region (CYL: P < 0.0001; PYL: P = 0.0117). However, only a general trend was found between g_s and

 Ψ_{pd} when means per region and fragmentation level were considered (Fig. 3a,b). This mismatch in the results could be due to the mixed models considered tree variability (random factor) while the means didn't separate that effect.

Photochemical efficiency of leaves (predawn, midday and their difference) significantly depended on water stress (Fig. 3c–h). For a given Ψ_{pd} , differences in photochemical efficiency of leaves among fragmentation levels, i.e. variability among fragmentation levels, were higher at predawn than at midday (Fig. 3c–f). Besides, F_v/F_m and $\Delta F_v/F_m$ showed slightly higher sensibility to Ψ_{pd} in CYL than in PYL at high water stress ($\Psi_{pd} < -3$ MPa).

Regarding ground fluorescence, it suffered a strong regulation by predawn water potential which was not observed on the maximum fluorescence. Predawn ground fluorescence (F_{opd}) was positively affected by water stress during LSu in both kind of leaves (P = 0.0422 (CYL); P = 0.0203 (PYL)) (Fig. 4). Besides, water stress accentuated the



difference between predawn and midday values (ΔF_{o}) only in PYL (Fig. 4).

4. Discussion

Our results support the idea that fragmentation can attenuate negative drought impacts on tree functioning, improving water status of trees even during intense drought periods. In these conditions, severe water stress limits gas exchange and reduces growth rates of oaks in dense stands (Coll et al., 2013; Moreno and Cubera, 2008). There are large discrepancies in previous studies about the water potential at which *Quercus ilex* losses the 50 % of its hydraulic conductivity (P_{50}) (Lobo et al., 2018; Martin-StPaul et al., 2014). These discrepancies are mainly due to the different methods used to calculate the vulnerability to embolism (Cochard et al., 2013). Recent studies indicate that P_{50} for Q. ilex is not higher than -5.5 MPa (Urli et al., 2014) and could be lower than -7.1 MPa (Lobo et al., 2018). These water potentials were not reached by our study trees even during the most severe drought conditions. Only trees from interiors in the southern region reached water potentials between -4 MPa and -5 MPa which can produce around 12 % of hydraulic conductivity loss (-4.3 ± 0.1 MPa; northern region: -3.2 ± 0.01 MPa) (Lobo et al., 2018; Urli et al., 2014). At first, these levels of water stress do not compromise the hydraulic wellfunction of trees from the interior fragments, but it compromises acorn production (Alejano et al., 2008; Morán-López et al., 2016). However, those from nearby small forest fragments may maintain their entire functionality, since those trees only suffered moderate water stress

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Fig. 2. In current-year leaves (CYL), measurements of stomatal conductance (g_{s} , mmol s⁻¹ m⁻²) (a,b) and predawn and midday maximum photochemical efficiency of photosystem II (F_v/F_{mpd} and F_v/F_{mpd} respectively; unitless) (c,d and e,f respectively). Bars correspond to measurements (mean ± SE) performed in different fragmentation levels (Interior, Edge and Small), in early (ESu) and late (LSu) summer of both study years (dry and wet). Left and right panels correspond to the northern and southern region, respectively. For each year and period *different letters* indicate statistically significant differences between fragmentation levels at P < 0.05.

(northern region: -2.5 ± 0.14 MPa; southern region: -2.8 ± 0.12 MPa). Thus, differences on tree water stress according to forest fragmentation level were translated to functional differences in the regulation of leaf water losses and photochemistry.

Photoinhibition in trees seems to have started between -2.5 MPa and -3 MPa, and was triggered at around -3.5 MPa, when the maximum photochemical efficiency of photosystem II (F_v/F_m) decreased significantly, and the ground fluorescence (F_{0}) increased. High levels of F_{0} can be due to inactivation and increase of non-functional reaction centers of the PSII (Bussotti, 2004), since the difference between predawn and midday photochemical efficiency ($\Delta F_v/F_m$) increased sharply at around -3 MPa. This evidenced photochemical damages in holm oaks from interiors and edges in the driest conditions, and chronic photoinhibition inferred from the increase in Fo (Werner et al., 2002) when water predawn potential decreased below -4 MPa. On the contrary, oaks located in small forest fragments always maintained high values of maximum photochemical efficiency (midday Fv/Fm around 0.8; while < 0.7 in interiors and edges), and did not show chronic photoinhibition. Nevertheless, photochemistry showed a relatively high resistance to drought in Quercus ilex (Ogaya and Peñuelas, 2003; Ogaya et al., 2011), probably linked to a high photoprotective system as observed in Q. suber, a close related species (Garcia-Plazaola et al., 1997). Only under the most stressful conditions in the southern region, and during dry summer months when Ψ_{pd} drop below -3 MPa, leaf photochemistry was altered in terms of permanent photo-inhibition (i.e. decrease of F_v/F_m , and in the difference of F_v/F_m between predawn and midday, or increase in F_0).



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Fig. 3. Effects of predawn water potential (Ψ_{pd}, MPa) on tree functioning. In particular, on stomatal conductance (g_s , mmol s^{-1} m⁻²; a,b), the predawn (F_v/F_{mpd} ; c,d) and midday $(F_v/F_{mmd}; e,f)$ maximum photochemical efficiency of photosystem II and the difference between both variables $(\Delta F_{\nu}/F_m; g,h)$. Measurements of ecophysiological variables were performed in current-year (CYL; left panels) and previous-year (PYL; right panels) leaves. Points depict mean ± SE per year, fragmentation level (I: interior; E: edge; S: small) and site. Ψ_{pd} was measured in PYL. All measurements were done in late summer (LSu). Note that different scales are shown for F_v/F_{mpd} and F_v/F_{mmd} to allow a better display. n.s. no significant relationships were found.

In both years, values of stomatal conductance reported in the fragmented forests were similar to those found under wetter conditions $(125-200 \text{ mmol m}^2 \text{ s}^{-1} \text{ (mean)}; \text{ Tognetti et al., } 1998; \text{ Nardini et al., }$ 2000) and in low density stands (Infante et al., 2003). In contrast, under the same climatic conditions in forest interiors and edges, stomatal conductance dropped to values comparable to those previously observed under intense drought events in high density stands (50-75 mmol m² s⁻¹ (mean); Peñuelas et al., 1998; Tognetti et al., 1998; Moreno and Cubera, 2008) and in plants subjected to severe drought conditions (Forner et al., 2018; Peguero-Pina et al., 2018). It is important to note, however, that the effects of water stress on stomatal conductance were weaker than on photochemical efficiency. Even though forest fragmentation influenced the effects of drought, we only detected a clear relationship between conductance and predawn water potentials in forest interiors. The high sensitivity of tree stomatal conductance to local vapor pressure deficit (Barbeta et al., 2012; Oren et al., 1999) could increase inter-individual variability and explain the non-significant responses of stomatal conductance to water stress found when means per fragmentation level and region were considered. However, when this variability is considered in the statistical models, the general relationship between stomatal conductance and water stress is significant. Therefore, tree stomatal conductance is affected by water stress at least during late summer conditions.

Fragmentation also determined leaf structural traits, but only under the driest conditions. Although a low responsiveness of SLA has been claimed for drought resistant species (Poorter et al., 2009) or observed in the short term from drought experiments (Martin-St Paul et al., 2012). We found high specific leaf area and low leaf dry matter content in leaves of trees from small fragments submitted to the lowest water stress. Higher SLA is generally correlated with lower inter-tree competition effects, higher photosynthetic rate and higher soil nutrients (Kunstler et al., 2015; Pérez-Harguindeguy et al., 2013). Thus, low soil



moisture availability affects anatomical plasticity of leaves by decreasing the SLA across geographical gradients differing in dryness (Castro-Diez et al., 1997), or even from adaptive processes to local dry conditions (Ramírez-Valiente et al., 2010). Differences between previous- and current-year leaves were stronger under drier conditions, although it depended on the study variable. Such differences may lay on the variability in leaf photoprotection within the same tree, which is influenced by leaf age (Camarero et al., 2012). In general, previous-year leaves showed higher sensibility to fragmentation and drought effects, which explains the early shedding of old leaves in extremely dry years in these sites and conditions.

The attenuation of drought effects on tree functioning in small forest fragments was reinforced under drier conditions (i.e. in the southern region and the driest year), confirming that the positive effect of fragmentation on tree functioning increases during severe drought. Our data support the general idea that reductions of inter-individual competition for water is important to maintain growth, function and sustainability under increasing arid conditions for holm oak stands (Barbeta and Peñuelas, 2016; Di Matteo et al., 2017; Gavinet et al., 2019). The improved environmental conditions for trees of small forest fragments could have been caused by relaxed tree to-tree competition for water resources at forest edges (Morán-López et al., 2016; Moreno and Cubera, 2008). Values of canopy cover around focal trees in small forest fragments are similar to those reported in low-density stands $(\approx 30 \%)$, where oaks show enhanced physiological conditions under intense droughts. Similarly, to our results, they are able to operate in water potentials above safety limits, maintaining high sap flow and carbon fixation rates during summer (Cubera and Moreno, 2007; Moreno and Cubera, 2008). In this line, selective thinning has been proposed as a management strategy to reduce forest sensitivity to drought and drought-induced mortality in holm oak stands (Gavinet et al., 2019; Rodríguez-Calcerrada et al., 2011). Nonetheless, in fragmented landscapes, changes in habitat quality could have also contributed to a better water status of trees (Ewers and Didham, 2006; Flores-Rentería et al., 2016). In fact, matrix loadings have been linked to higher fertility and water-holding capacity of soils from small holm oak fragments of our study area (Flores-Rentería et al., 2018b). Moreover, some nutrients deposition coming from the agricultural matrix has been detected in our study site (Flores-Rentería et al., 2016). Even though the surrounding croplands were not irrigated and trees did not **Fig. 4.** Effects of predawn water potential (Ψ_{pd}, MPa) on ground fluorescence measured at predawn $(F_{opd}; left panels)$, and on the difference between predawn (F_{opd}) and midday values (F_{omd}) $(\Delta F_o; right panels)$. Measurements were performed in current-year (CYL; a,c) and previous-year (PYL; b,d) leaves. Points depict mean \pm SE per year, fragmentation level (I: interior; E: edge; S: small) and site. Ψ_{pd} was measured in PYL. Data was measured in late summer (LSu).

have extra water availability, water infiltration in ploughed soils could have reduced water stress of holm oaks (Carevic et al., 2010), due to their ability to exploit water in soil interspaces and rock fractures (Moreno and Cubera, 2008; Barbeta and Peñuelas, 2016). Therefore, sometimes, the effect of surrounding matrix cannot be isolated because it is interconnected with the direct effect of fragmentation, creating a complex cascade of causal-effect relations which modify soil environment conditions (such as microbial diversity) (Flores-Rentería et al., 2018a,b). Nonetheless, despite differences in habitat quality among fragments, we expect that its effects on tree ecophysiology are much lower than the ones produced by intraspecific competition due to fragmentation. Irrespectively of the underlying causes, our results show that attenuation of water stress in fragmented areas enabled oaks located at small fragments to avoid photochemical damage, and under intense drought periods maintain high stomatal conductance. The fact that local environmental conditions such as fragmentation overrode drought effects emphasizes the importance of local-scale studies when addressing the potential impacts of such drivers. In addition, the concomitant effect of fragmentation and drought reinforces the idea that studies addressing the combined effects of different drivers are critical to improve global change projections in Mediterranean areas (Doblas-Miranda et al., 2017).

Overall, our results show that, unlike forest interiors and edges, trees located in small forest fragments are able to maintain high functionality during summer, even when drought intensity is severe. The implications of these patterns on the vulnerability of holm oaks to cavitation must be considered in future studies in order to evaluate the potential long-term synergic effects between fragmentation and drought. Furthermore, the fact that fragmentation effects were more important under increased aridity point out that its role as a drought modulator may increase in the future since more intense and frequent drought events are expected (IPCC, 2014). Our results from the fragmented holm oak landscapes study disagrees with the usually assumed expectation of negative effects of habitat fragmentation on plant performance (Fahrig, 2003), e.g. limiting the plasticity and evolutionary potential of plants under climate change (Matesanz and Valladares, 2014), disruption of plant-animal interactions (Leimu et al., 2010), less effective seed dispersal (Morán-López et al., 2015), and changes in the landscape diversity of soil microbial communities (Flores-Rentería et al., 2016), among others. In line with our results, increased acorn

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production (Morán-López et al., 2016) and enhanced water and nutrient availabilities in the soil (Flores-Rentería et al., 2015) have been reported for isolated or small holm oak patches included in agricultural matrixes. Nonetheless, we must be cautious when extrapolating these positive impacts in plant performance to population dynamics because isolated populations of holm oaks could also suffer from negative impacts, for example on regeneration or on the adaptability from genetic drift. Long-term positive effects of forest fragmentation on holm oak woodlands will depend on the balance between a higher vegetative performance of adult trees in isolated or small forest fragments and a successful recruitment at the local and regional scales.

5. Conclusions

Forest fragmentation attenuated the negative impacts of drought on holm oak functioning and this attenuation was reinforced during drier environmental conditions, which gives fragmented forests a better opportunity to withstand intense droughts already observed under the ongoing climate change in the Mediterranean region. Therefore, fragmentation could maintain tree functioning and buffer the water stress endured by trees under severe droughts. This could be much more relevant in the next decade when more recurrent and intense droughts are expected. Furthermore, these better conditions for vegetative growth in small fragments would explain partially the current increase of woody vegetation in adjacent abandoned agricultural fields. However, the observed carry-over effects on Mediterranean forests caused by frequent and long droughts spread the doubt whether fragmentation may attenuate the effects of several consecutive severe droughts.

Author contributions

IA and FV conceived the ideas and designed methodology. AF, TML and DFR collected the data. AF analyzed the data and wrote a first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declaration of Competing Interest

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2020. 104001.

References

- Alejano, R., Tapias, R., Fernández, M., Torres, E., Alaejos, J., Domingo, J., 2008. Influence of pruning and the climatic conditions on acorn production in holm oak (Quercus ilex L.) dehesas in SW Spain. Ann. For. Sci. 65, 209.
- Aparicio, A., Hampe, A., Fernández-Carrillo, L., Albaladejo, R.G., 2012. Fragmentation and comparative genetic structure of four mediterranean woody species: complex interactions between life history traits and the landscape context. Divers. Distrib. 18, 226–235.
- Barbeta, A., Peñuelas, J., 2016. Sequence of plant responses to droughts of different timescales: lessons from holm oak (Quercus ilex) forests. Plant Ecol. Divers. 9, 321–338.
- Barbeta, A., Ogaya, R., Peñuelas, J., 2012. Comparative study of diurnal and nocturnal sap flow of *Quercus ilex* and *Phillyrea latifolia* in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). Trees 26, 1651–1659.
- Barbeta, A., Mejía-Chang, M., Ogaya, R., Voltas, J., Dawson, T.E., Peñuelas, J., 2015. The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. Glob. Change Biol. 21, 1213–1225.
- Bolhar-Nordenkampf, H.R., Long, S.P., Baker, N.R., Oquist, G., Schreiber, U., Lechner, E.G., 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3, 497–514.
- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann. For. Sci. 63, 625–644.
- Briantais, J.-M., Dacosta, J., Goulas, Y., Ducruet, J.-M., Moya, I., 1996. Heat stress induces in leaves an increase of the minimum level of chlorophyll fluorescence, Fo: a timeresolved analysis. Photosynth. Res. 48, 189–196.
- Bussotti, F., 2004. Assessment of stress conditions in Quercus ilex L. leaves by O-J-I-P chlorophyll a fluorescence analysis. Plant Biosyst. 138, 101–109.
- Camarero, J.J., Lloret, F., Corcuera, L., Peñuelas, J., Gil-Pelegrín, E., 2004. Cambio global y decaimiento del bosque. Ecología del bosque mediterráneo en un mundo cambiante. pp. 397–423.
- Camarero, J.J., Olano, J.M., Arroyo Alfaro, S.J., Fernández-Marín, B., Becerril, J.M., García-Plazaola, J.I., 2012. Photoprotection mechanisms in Quercus ilex under contrasting climatic conditions. Flora — Morphol. Distrib. Funct. Ecol. Plants 207, 557–564.
- Carevic, F.S., Fernández, M., Alejano, R., Vazquez-Pique, J., Tapias, R., Corral, E., Domingo, J., 2010. Plant water relations and edaphoclimatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp ballota) open woodland. Agrofor. Syst. 78, 299–308.
- Castro-Diez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martinez, M., Montserrat-Martí, G., 1997. Leaf morphology and leaf chemical composition in three Quercus (Fagaceae) species along a rainfall gradient in NE Spain. Trees 11, 127–134.
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. Ann. Bot. 89, 907–916.
- Cochard, H., Badel, E., Herbette, S., Delzon, S., Choat, B., Jansen, S., 2013. Methods for measuring plant vulnerability to cavitation: a critical review. J. Exp. Bot. 64 (15), 4779–4791.
- Colangelo, M., Camarero, J.J., Battipaglia, G., Borghetti, M., De Micco, V., Gentilesca, T., Ripullone, F., 2017. A multi-proxy assessment of dieback causes in a Mediterranean oak species. Tree Physiol. 37, 617–631.
- Coll, M., Peñuelas, J., Ninyerola, M., Pons, X., Carnicer, J., 2013. Multivariate effect gradients driving forest demographic responses in the Iberian Peninsula. For. Ecol. Manage. 303, 195–209.
- Corcuera, L., Camarero, J., Gil-Pelegrín, E., 2004. Effects of a severe drought on Quercus ilex radial growth and xylem anatomy. Trees 18, 83–92.
- Cubera, E., Moreno, G., 2007. Effect of single *Quercus ilex* trees upon spatial and seasonal changes in soil water content in dehesas of central western Spain. Ann. For. Sci. 64, 355–364.
- Di Matteo, G., Nardi, P., Fabbio, G., 2017. On the use of stable carbon isotopes to detect the physiological impact of forest management: the case of Mediterranean coppice woodland. For. Ecol. Manage. 389, 158–166.
- Díaz, M., Santos, T., Tellería, J., 1999. Effects of forest fragmentation on the winter body condition and population parameters of an habitat generalist, the wood mouse Apodemus sylvaticus: a test of hypotheses. Acta Oecol. 20, 39–49.
- Doblas-Miranda, E., Alonso, R., Arnan, X., Bermejo, V., Brotors, L., de las Heras, J., Estiarte, M., Hódar, J.A., Llorens, P., Lloret, F., López-Serrano, F.R., Martínez-Vilalta, J., Moya, D., Peñuelas, J., Pino, J., Rodrigo, A., Roura-Pascual, N., Valladares, F., Vilà, M., Zamora, R., Retana, J., 2017. A review of the combination among global change factors in forests, shrublands and pastures of the Mediterranean Region: beyond drought effects. Glob. Planet. Change 148, 42–54.
- Ewers, R.M., Didham, R.K., 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biol. Rev. 81, 117–142.
- Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34, 487–515.
- Fernández, C., Acosta, F.J., Abellá, G., López, F., Díaz, M., 2002. Complex edge effect fields as additive processes in patches of ecological systems. Ecol. Modell. 149, 273–283.
- Flexas, J., Diaz-Espejo, A., Gago, J., Gallé, A., Galmés, J., Gulías, J., Medrano, H., 2014. Photosynthetic limitations in Mediterranean plants: a review. Environ. Exp. Bot. 103, 12–23.
- Flores-Rentería, D., Curiel Yuste, J., Rincón, A., Brearley, F.Q., García-Gil, J.C., Valladares, F., 2015. Habitat fragmentation can modulate drought effects on the plant-soil-microbial system in Mediterranean holm oak (Quercus ilex) forests. Microb. Ecol. 69, 798–812.
- Flores-Rentería, D., Rincón, A., Valladares, F., Curiel Yuste, J., 2016. Agricultural matrix affects differently the alpha and beta structural and functional diversity of soil microbial communities in a fragmented Mediterranean holm oak forest. Soil Biol.

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Biochem. 92, 79-90.

- Flores-Rentería, D., Curiel Yuste, J., Valladares, F., Rincón, A., 2018Ea. Soil legacies determine the resistance of an experimental plant-soil system to drought. Catena 166, 271-278.
- Flores-Bentería, D., Rincón, A., Morán-López, T., Heres, A.-M., Pérez-Izquierdo, J., Valladares, F., Curiel Yuste, J., 2018Eb. Habitat fragmentation is linked to cascading effects on soil functioning and CO2 emissions in Mediterranean holm-oak-forests. PeerJ 6, e5857.
- Forner, A., Aranda, I., Granier, A., Valladares, F., 2014. Differential impact of the most extreme drought event over the last half century on growth and sap flow in two coexisting Mediterranean trees. Plant Ecol. 215, 703-719.
- Forner, A., Valladares, F., Aranda, I., 2018. Mediterranean trees coping with severe drought: avoidance might not be safe. Environ. Exp. Bot. 155, 529-540.
- García, F.J., Díaz, M., de Alba, J.M., Alonso, C.L., Carbonell, R., Carrión, M.L., Monedero, C., Santos, T., 1998. Edge effects and patterns of winter abundance of wood mice Apodemus sylvaticus in Spanish fragmented forests. Acta Theriol. 43, 255-262.
- Garcia-Plazaola, J.I., Faria, T., Abadia, J., Abadia, A., Chaves, M.M., Pereira, J.S., 1997 Seasonal changes in xanthophyll composition and photosynthesis of cork oak (Quercus suber L.) leaves under mediterranean climate. J. Exp. Bot. 48, 1667–1674.
- García-Valdés, R., Svenning, J.-C., Zavala, M.A., Purves, D.W., Araújo, M.B., 2015. Evaluating the combined effects of climate and land-use change on tree species distributions. J. Appl. Ecol. 52, 902-912.
- Garnier, E., Shipley, B., Roumet, C., Laurent, G., 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Funct. Ecol. 15, 688-695.
- Gavinet, J., Ourcival, J.M., Limousin, J.M., 2019. Rainfall exclusion and thinning can alter the relationships between forest functioning and drought, New Phytol. 223, 1267-1279.
- Gentilesca, T., Camarero, J., Colangelo, M., Nole, A., Ripullone, F., 2017. Drought-induced oak decline in the western Mediterranean region: an overview on current evidences, mechanisms and management options to improve forest resilience. iForest Biogeosci. For. 10, 796-806.
- Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A., Nicholls, A.O., Orrock, J.L., Song, D.-X., Townshend, J.R.,
- 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Sci. Adv. 1. Hereş, A.-M., Kaye, M.W., Granda, E., Benavides, R., Lázaro-Nogal, A., Rubio-Casal, A.E., Valladares, F., Curiel Yuste, J., 2018. Tree vigour influences secondary growth but not responsiveness to climatic variability in Holm oak. Dendrochronologia 49, 68-76.
- Infante, J.M., Domingo, F., Fernández Alés, R., Joffre, R., Rambal, S., 2003. Quercus ilex transpiration as affected by a prolonged drought period. Biol. Plant. 46, 49–55.
- IPCC, 2014. Climate change 2014: synthesis report. In: Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.
- Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.
 Kunstler, G., Falster, D., Coomes, D.A., Hui, F., Kooyman, R.M., Laughlin, D.C., Poorter, L., Vanderwel, M., Vieilledent, G., Wright, S.J., Aiba, M., Baraloto, C., Caspersen, J., Cornelissen, J.H.C., Gourlet-Fleury, S., Hanewinkel, M., Herault, B., Kattge, J., Kurokawa, H., Onoda, Y., Peñuelas, J., Poorter, H., Uriarte, M., Richardson, S., Ruiz-Benito, P., Sun, I.F., Ståhl, G., Swenson, N.G., Thompson, J., Westerlund, B., Wirth, C., Zavala, M.A., Zeng, H., Zimmerman, J.K., Zimmermann, N.E., Westoby, M., 2015. Plant functional traits have globally consistent effects on competition. Nature 529,
- 204 Leimu, R., Vergeer, P., Angeloni, F., Ouborg, N.J., 2010. Habitat fragmentation, climate change, and inbreeding in plants. Ann. N. Y. Acad. Sci. 1195, 84–98. Lloret, F., Siscart, D., Dalmases, C., 2004. Canopy recovery after drought dieback in holm-
- oak Mediterranean forests of Catalonia (NE Spain). Glob. Change Biol. 10, 2092-2099.
- Lobo, A., Torres-Ruiz, J.M., Burlett, R., Lemaire, C., Parise, C., Francioni, C., Truffaut, L., Tomášková, I., Hansen, J.K., Kjær, E.D., Kremer, A., 2018. Assessing inter- and intraspecific variability of xylem vulnerability to embolism in oaks. For. Ecol. Manage 424, 53-61.
- Martin-St Paul, N.K., Limousin, J.-M., Rodríguez-Calcerrada, J., Ruffault, J., Rambal, S., Letts, M.G., Misson, L., 2012. Photosynthetic sensitivity to drought varies among populations of Quercus ilex along a rainfall gradient. Funct. Plant Biol. 39, 25-37.
- Martin-StPaul, N.K., Longepierre, D., Huc, R., Delzon, S., Burlett, R., Joffre, R., Rambal, S., Cochard, H., 2014. How reliable are methods to assess xylem vulnerability to cavitation? The issue of 'open vessel' artifact in oaks. Tree Physiol. 34 (8), 894–905.
 Matesanz, S., Valladares, F., 2014. Ecological and evolutionary responses of Mediterranean plants to global change. Environ. Exp. Bot. 103, 53–67.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence-a practical guide. J. Exp.
- Bot. 51, 659-668. Morán-López, T., Fernández, M., Alonso, C.L., Flores-Rentería, D., Valladares, F., Díaz, M.,
- 2015. Effects of forest fragmentation on the oak-rodent mutualism. Oikos 124, 1482-1491.
- Morán-López, T., Forner, A., Flores-Rentería, D., Díaz, M., Valladares, F., 2016. Some positive effects of the fragmentation of holm oak forests: attenuation of water stress and enhancement of acorn production. For. Ecol. Manage. 370, 22-30.
- Moreno, G., Cubera, E., 2008. Impact of stand density on water status and leaf gas exchange in Quercus ilex. For. Ecol. Manage. 254, 74-84.
- Munné-Bosch, S., 2007. Aging in perennials. Crit. Rev. Plant Sci. 26, 123-138. Nardini, A., Salleo, S., Lo Gullo, M.A., Pitt, F., 2000. Different responses to drought and freeze stress of Quercus ilex L. growing along a latitudinal gradient. Plant Ecol. 148, 139-147.
- Ogaya, R., Peñuelas, J., 2003. Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest. Flora 198, 132-141.

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- Ogaya, R., Peñuelas, J., Asensio, D., Llusià, J., 2011. Chlorophyll fluorescence responses to temperature and water availability in two co-dominant Mediterranean shrub and tree species in a long-term field experiment simulating climate change. Environ. Exp. Bot. 73, 89–93.
- Opdam, P., Wascher, D., 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. Biol. Conserv. 117, 285-297.
- Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N., Schäfer, K.V.R., 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant Cell Environ. 22, 1515-1526.
- Ortego, J., Bonal, R., Muñoz, A., 2010. Genetic consequences of habitat fragmentation in long-lived tree species; the case of the Mediterranean holm oak (Ouercus ilex L.). J. Hered. 101, 717–726.
- Ortego, J., Bonal, R., Muñoz, A., Aparicio, J.M., 2014. Extensive pollen immigration and no evidence of disrupted mating patterns or reproduction in a highly fragmented holm oak stand. J. Plant Ecol. 7, 384-395.
- Parmesan, C., Burrows, M.T., Duarte, C.M., Poloczanska, E.S., Richardson, A.J., Schoeman, D.S., Singer, M.C., 2013. Beyond climate change attribution in con-servation and ecological research. Ecol. Lett. 16, 58–71.
- Peguero-Pina, J., Mendoza-Herrer, Ó., Gil-Pelegrín, E., Sancho-Knapik, D., 2018. Cavitation limits the recovery of gas exchange after severe drought stress in holm oak (Quercus ilex L.). Forests 9, 443.
- Peñuelas, J., Filella, I., Llusià, J., Siscart, D., Piñol, J., 1998. Comparative field study of spring and summer leaf gas exchange and photobiology of the mediterranean trees Quercus ilex and Phillyrea latifolia. J. Exp. Bot. 49, 229–238.
- Peñuelas, J., Lloret, F., Montoya, R., 2001. Severe drought effects on Mediterranean woody flora in Spain. For. Sci. 47, 214-218.
- Woody Holt an Opani. Fol. Cd. 7, 217–210.
 Pérez-Harguindeguy, N., Díaz, G., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S., Cornelissen, J.H.C., 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61, 167–234.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2007. The R Development Core Team (2000) Nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-108.
- Poorter, H., Niinemets, U., Poorter, L., Wright, I.J., Villar, R., 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182, 565-588
- Quero, J.L., Sterck, F.J., Martinez-Vilalta, J., Villar, R., 2011. Water-use strategies of six co-existing Mediterranean woody species during a summer drought. Oecologia 166, 45-57.
- R Core Team, 2016. R: A Language and Environmental for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Ramírez-Valiente, J.A., Sánchez-Gómez, D., Aranda, I., Valladares, F., 2010. Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. Tree Physiol. 30, 618-627.

Rasband, W.S., 2009. ImageJ. National Institutes of Health, Bethesda, MD, Bethesda, MD. Rodríguez-Calcerrada, J., Pérez-Ramos, I.M., Ourcival, J.M., Limousin, J.M., Joffre, R.,

Rambal, S., 2011. Is selective thinning an adequate practice for adapting Quercus ilex coppices to climate change? Ann. For. Sci. 68, 575-585.

Rodríguez-Calcerrada, J., Li, M., López, R., Cano, F.J., Oleksyn, J., Atkin, O.K., Pita, P., Aranda, I., Gil, L., 2017. Drought-induced shoot dieback starts with massive root xylem embolism and variable depletion of nonstructural carbohydrates in seedlings of two tree species. New Phytol. 213, 597–610.

Santos, T., Tellería, J., 1997. Vertebrate predation on Holm Oak, Quercus ilex, acorns in a fragmented habitat: effects on seedling recruitment. For. Ecol. Manage. 98, 181-187.

Santos, T., Tellería, J.L., 1998. Efectos de la fragmentación de los bosques sobre los vertebrados de las mesetas ibéricas. Organismo Autónomo Parques Nacionales. Ministerio Medio Ambiente, Madrid, Spain.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science 148, 339–346.

Tognetti, R., Longobucco, A., Raschi, A., 1998. Vulnerability of xylem to embolism in relation to plant hydraulic resistance in Quercus pubescens and Quercus ilex co-occurring in a Mediterranean coppice stand in central Italy. New Phytol. 139, 437–447.

Travis, J.M., 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proc. Biol. Sci. R. Soc. 270, 467-473.

- Urli, M., Porté, A.J., Cochard, H., Guengant, Y., Burlett, R., Delzon, S., 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol. 33, 672-683.
- Urli, M., Lamy, J.-B., Sin, F., Burlett, R., Delzon, S., Porté, A.J., 2014. The high vulnerability of Quercus robur to drought at its southern margin paves the way for Quercus ilex. Plant Ecol. 216, 177-187.
- Valladares, F., Benavides, R., Rabasa, S., Pausas, J., Paula, S., Simonson, W., Díaz, M., 2014a. Global change and Mediterranean forests: current impacts and potential responses. Forests and Global Change. pp. 47-75.
- Valladares, F., Flores-Rentería, D., Forner, A., Morán-López, T., Díaz, M., 2014b. Influencia de la fragmentación y el clima en procesos clave para la regeneración del encinar. Ecosistemas 23, 37-47.
- Werner, C., Correia, O., Beyschlag, W., 2002. Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem. Funct. Plant Biol. 29, 999-1011. WRB, I.W.G., 2007. World Reference Base for Soil Resources 2006, First Update 2007.
- FAO. W.S.R. Reports, Rome, Italy, pp. 103-116.
- Zuur, A., Ieno, E., Smith, G., 2007. Analysing Ecological Data. New York. .