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Original article

**Non-structural carbohydrate dynamics associated with drought-induced die-off in  
woody species of a shrubland community**

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Running title: NSC dynamics in shrubland under drought-induced die-off

# 1    **Abstract**

## 2•    *Background and Aims*

3•

4    The relationship between plant carbon economy and drought responses of co-occurring  
5    woody species can be assessed by comparing carbohydrate (C) dynamics following drought  
6    and rain periods, relating these dynamics to species' functional traits. We studied nine woody  
7    species coexisting in a continental Mediterranean shrubland that experienced severe drought  
8    effects followed by rain.

9

## 10•    *Methods*

11•

12    We measured total non-structural carbohydrates (NSC) and soluble sugars (SS) in roots and  
13    stems during drought and after an autumn rain pulse in plants exhibiting leaf loss and in  
14    undefoliated ones. We explored whether their dynamics were related to foliage recovery and  
15    functional traits (height H; specific leaf area SLA; wood density WD).

16

## 17•    *Key Results*

18•

19    During drought, NSC concentrations were overall lower in stems and roots of plants  
20    experiencing leaf loss, while SS decreases were smaller. Roots had higher NSC  
21    concentrations than stems. After the rain, NSC concentrations continued to decrease, while  
22    SS increased. Green foliage recovered after rain, particularly in plants previously  
23    experiencing higher leaf loss, independent of NSC concentrations during drought. Species  
24    with lower WD tended to have more SS during drought and lower SS increases after rain. In  
25    low-WD species, plants with severe leaf loss had lower NSC relative to undefoliated ones.  
26    No significant relationship was found between H or SLA and C content or dynamics.

27

1• *Conclusions*

2

3 Our community-level study reveals that, while responses were species-specific, C stocks  
4 overall diminished in plants affected by prolonged drought and did not increase after a pulse  
5 of seasonal rain. SS showed faster dynamics than NSC. We found limited depletion of SS,  
6 consistent with their role in basal metabolic, transport, and signalling functions. In a scenario  
7 of increased drought under climate change, NSC stocks in woody plants are expected to  
8 decrease differentially in coexisting species, with potential implications for their adaptive  
9 abilities and community dynamics.

10

11 **Keywords:** climate change, canopy dieback, die-off, drought, Mediterranean, non-structural  
12 carbohydrates, shrubland, plant functional traits, soluble sugars, wood density.

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## 1   **Introduction**

2  
3   Climate change is increasing aridity in many parts of the world (Dai, 2012) and this trend is  
4   predicted to continue in the near future, particularly in the Mediterranean Basin (Gao et al.  
5   2006; Giannakopoulos *et al.*, 2009). As a result, Mediterranean plant communities are  
6   experiencing longer periods of drought, punctuated by precipitation events that usually  
7   follow a seasonal pattern (Sarris et al., 2007; Briffa et al., 2009). The role of these pulses is  
8   extremely relevant since they could determine the resilience of plant communities under  
9   climate change (Lloret *et al.*, 2012). Although drought events are known to lead to vegetation  
10   die-off and canopy dieback in many regions of the world (Allen *et al.*, 2010; Greenwood *et*  
11   *al.*, 2017), most studies have dealt with acute episodes of heatwaves and/or water deficit  
12   (e.g., Breshears *et al.*, 2005; Galiano *et al.*, 2011; Lloret *et al.*, 2016; Venturas *et al.*, 2016) or  
13   prolonged drought periods (e.g., Sapes *et al.*, 2017) and the more common scenario of  
14   drought periods punctuated by pulses of rain has not been explored in any depth.

15  
16   Although most of our knowledge on vegetation die-off has focused on forests, the  
17   phenomenon also occurs in shrublands (e.g. Palacio *et al.*, 2007; Lloret *et al.*, 2016; Sapes *et*  
18   *al.*, 2017), a type of ecosystem of great importance in terms of global extension, diversity,  
19   and ecosystem services (McKell, 1975; van Wilgen *et al.*, 1996; Petri *et al.*, 2015). The  
20   diversity of the woody species coexisting in many shrubland communities provides an  
21   opportunity to study the general processes involved in canopy dieback and the subsequent  
22   recovery across species, as well as relating the range of responses (Breshears *et al.*, 2005;  
23   Sapes *et al.*, 2017) to species' traits, life history strategies (Lloret *et al.*, 2016), drought  
24   tolerance (Rosas et al. 2013), and carbon (C) economy strategies (Klein *et al.*, 2014; Galiano  
25   *et al.*, 2017).

The mechanistic basis underlying drought-induced canopy dieback and eventual mortality has still not been completely identified, but there is agreement that it involves not only the impairment of the long-distance transport connecting the supply and demand of water and C but also alterations in the C balance (McDowell *et al.*, 2008; Sala *et al.*, 2010; McDowell *et al.*, 2011, 2013; Adams *et al.*, 2017). In terms of the C economy, plants distribute recent C assimilated by leaves (sources) into various C sinks such as growth, metabolic maintenance, storage, defence, export, and reproduction (Chapin *et al.*, 1990; Körner, 2003). It has been suggested that stored non-structural carbohydrates (NSC) are never fully depleted under average conditions because a certain concentration of soluble sugars (SS) is required to sustain immediate plant functions such as osmoregulation, transport, and signalling (Sala *et al.*, 2010; Hartmann and Trumbore, 2016; Martínez-Vilalta *et al.*, 2016). The storage component is of major importance for plant function and survival under stressful conditions (O'Brien *et al.*, 2014; Sala and Mencuccini, 2014). More particularly, stored NSC such as soluble sugars and starch provide a buffer against periods of water limitation in which stomatal closure prevents photosynthetic C uptake (McDowell *et al.*, 2008, 2011) as they provide the C that maintains basic metabolism and defence during drought stress (McDowell and Sevanto, 2010; Sala *et al.*, 2012). However, the numerous observational and experimental studies undertaken to date on NSC dynamics in response to drought have yielded mixed results, from decreases (Galiano *et al.*, 2011; Mitchell *et al.*, 2013; Sevanto *et al.*, 2014; Aguadé *et al.*, 2015) to increases in NSC concentrations, or no change at all (Sala and Hoch, 2009; Anderegg *et al.*, 2012; Gruber *et al.*, 2012; Hartmann *et al.*, 2013). There are also some contrasting results with respect to stems and roots (Klein *et al.*, 2014). Because photosynthesis is less sensitive to water stress than growth, increases in NSC are often observed during the early stages of drought (McDowell, 2011; Mitchell *et al.*, 2013;

Hagedorn *et al.*, 2016). Nevertheless, modelling and experimental research indicate that NSC reserves should eventually decline if drought lasts for sufficiently long periods (cf. above; McDowell *et al.*, 2013).

A species' C economy can also be related to growth and survival strategies associated with particular combinations of traits. Species' traits are in turn crucial to understanding how environmental filters such as water availability determine the assemblage of species in a local community (Shipley, 2010). A general, two-dimensional global spectrum of plant form and function has been identified, with the two main axes corresponding to leaf economics and plant size (Díaz *et al.*, 2016). The leaf economics spectrum highlights the trade-off between carbon and nutrient investments in leaf construction and the duration of returns on those investments. The spectrum runs from species with conservative leaf traits (i.e., long leaf lifespan, low specific leaf area (SLA), expensive construction, and slow returns on investments of carbon and nutrients) to species with acquisitive leaf traits (i.e., short lifespan, high SLA values, cheap construction, and fast returns on investment) (Wright *et al.*, 2004, Shipley *et al.*, 2006, Westoby *et al.*, 2013). Resource-conservative species are often considered to be more resistant to C loss and drought stress (Saura-Mas and Lloret, 2007), although a species' response often involves many interconnected traits (Anderegg *et al.*, 2012, McDowell *et al.*, 2011). More conservative species, for example, tend to have higher wood density and low growth rates (Chave *et al.*, 2009). Wood density is also a moderately good predictor of resistance to drought-induced embolism (Hacke *et al.*, 2001). The second main axis, plant size or maximum height, is related to life-history features and the ability to use resources over a continuum of colonization-exploitation (Díaz *et al.*, 2016). Accordingly, specific functional traits have been correlated with different responses to extreme drought episodes in coexisting species (e.g., Skelton *et al.*, 2015, Pivovarovoff *et al.*, 2016). In a recent

global synthesis, Greenwood *et al.* (2017) reported that species with higher SLA or lower wood density were more susceptible to drought-induced mortality. In Mediterranean shrublands from SW Spain, Lloret *et al.* (2016) found that species' resistance and resilience to drought was explained by water economy and recruitment-related traits, while another study in Californian chaparral highlighted the relevance of plant size (Venturas *et al.*, 2016), probably associated with deeper roots. However, the link between functional traits, plant carbon economy, and drought-induced die-off merits further exploration. To our knowledge, no studies have addressed C dynamics over a sequence of drought and subsequent rain at the plant community level, where different species with contrasting functional traits coexist.

Here, our main aim was to assess NSC dynamics in the aerial (stems) and underground organs (roots) of woody species coexisting in a Mediterranean semi-arid shrubland during a drought-induced dieback event and subsequent recovery after a rain episode. Canopy-dieback was estimated via the loss of leaves because leaf-shedding often occurs during drought episodes as an avoidance mechanism that maintains a favourable water status by reducing the transpiring leaf area (Dobbertin and Brang, 2001; Poyatos *et al.*, 2013; Jump *et al.*, 2017). We hypothesized that: (1) within species, plants with leaf loss exhibit lower NSC concentrations compared to undefoliated plants due to their reduced capacity to assimilate carbon over the duration of the drought episode; (2) SS are less variable than total NSC over time when plants with different levels of canopy dieback (leaf loss) are compared within species, reflecting the need to maintain some minimum concentrations of SS to sustain metabolism and osmoregulation; (3) an increase in green foliage following a post-drought rainy period is associated with a reduction in overall NSC concentrations because the carbon supply from the recovered canopy is insufficient to compensate for investment in new tissues or replenish stored reserves in a context of long-term drought, and (4) species characterized



by high growth potential (low wood density), less longevity and ability to compete (small size), or acquisitive leaf traits (high SLA) have lower and more variable NSC concentrations, reflecting a faster response to environmental variability (Trumbore *et al.*, 2015).

## Methods

### *Site description*

The study took place in the Barranco de Valcuerna (Monegros, central Ebro valley, Spain, 41°25'N, 0°4'E, Figure 1), at *ca.* 280 m above sea level (Figure 1a). The climate is continental Mediterranean with a mean annual temperature of 14.8 °C and mean annual precipitation of 390 mm; there are strong contrasts between the seasons, particularly with respect to temperatures, which range from mean values of 6.1 °C in January to 23.8°C in July. In recent decades, the combination of higher temperatures and low precipitation has increased aridity in the region (Figure 1b), resulting in a loss of vegetation cover (Vicente-Serrano *et al.*, 2012; Sapes *et al.*, 2017). However, the general trend towards increasing aridity is periodically interrupted by rainfall episodes that exceed the historical average precipitation, particularly in spring and autumn (Figure 1c).

The vegetation in this area is a mosaic of grassland, shrubland, and *Pinus halepensis* open forest (Braun-Blanquet and Bolós, 1957; Terradas, 1986). We focused our study on an open continental-Mediterranean shrubland dominated by suffrutescent shrubs (*Thymus vulgaris* L., *Lithospermum fruticosum* L., *Thymelaea tinctoria* (Pursh) Endl., *Helianthemum* sp. pl), small shrubs (*Rosmarinus officinalis* L., *Genista scorpius* (L.) DC., *Globularia alypum* L., *Cistus*

1 *libanotis* L., *Helianthemum syriacum* (Jacq.) Dum. Cours.), large shrubs (*Quercus coccifera*  
2 L., *Rhamnus lycioides* L., *Pistacia lentiscus* L.), with an occasional presence of short  
3 (*Juniperus phoenicea* L.) and taller trees (*Pinus halepensis* Mill). These species are generally  
4 found in a Mediterranean climate, although in this particular area some, such as *J. phoenicea*  
5 and *Q. coccifera*, are close to their tolerance limits with respect to aridity and low winter  
6 temperatures. This vegetation develops on leptosols over marls and limestones that are rich in  
7 gypsum.

#### 10 ***Recording field canopy dieback***

12 One particularly severe drought period lasted from 2009 to summer 2012 (Figure 1 B, C).  
13 This drought triggered widespread leaf loss (canopy dieback) in most woody species in the  
14 community. A pulse of rainfall occurred in autumn 2012, although temperatures remained  
15 above average and the drought persisted (Sapes *et al.*, 2017), as indicated by the Standardized  
16 Precipitation-Evapotranspiration Index (SPEI) (Vicente-Serrano et al. 2010). We selected two  
17 sampling times: April 2012, at the peak of the drought period, and February 2013, which  
18 followed the autumn rainy season.

20 Adults from nine woody species were selected according to the following criteria: i)  
21 perennial species that comprise a wide range of life forms, from suffruticose and small shrubs  
22 to trees; ii) species amenable to reliable visual estimates of drought-induced leaf loss; and iii)  
23 species comprising a wide range of leaf morphologies and wood densities. The species used  
24 in this study were *P. halepensis* (Ph), *J. phoenicea* (Jp), *Q. coccifera* (Qc), *R. lycioides* (Rl),  
25 *R. officinalis* (Ro), *L. fruticosum* (Lf), *G. scorpius* (Gs), *T. tinctoria* (Ti), *T. vulgaris* (Th)

(Table 1).

Visual estimates of green canopy level were considered a proxy for species' response to drought. This method is standard practice for measures of drought-induced impact on ecosystems dominated by woody vegetation (e.g., Nakajima *et al.*, 2011; Carnicer *et al.*, 2011; Galiano *et al.*, 2011; Bohemer *et al.*, 2013). In order to facilitate comparisons between species that may exhibit different leaf phenologies, the green canopy was estimated by considering the percentage of young, thin tips of branches with no signs of decay and regularly holding up leaves (as well as checking recent leaf scars in species that drop leaves early after unfolding, such as *G. scorpius*). Thus, although we considered the current levels of leaf loss, these also reflected the drought experienced in the study area in the previous years (Fig. 1 B, C). The visual estimates of green canopy in this community correlated well with the actual amounts of green leaves (leaves to branch weight ratio) (see Sapes *et al.*, 2017 for a more detailed description of green canopy estimates). Individuals with signs of decay prior to the drought period (stumps, decomposing stems, branches with no thin tips or buds) were excluded from the sampling. Then, for each species, we selected twenty undefoliated plants (> 80% green canopy, except in *R. lycioides* and *L. fruticosum*, in which these values were > 70 and 40%, respectively, because regardless of drought they commonly show tips of branches with no leaves) and twenty plants affected by canopy dieback (< 50% green canopy, except in *L. fruticosum*, in which this value was set to 25%, as explained above) and we categorized them as "undefoliated" or with "leaf loss", respectively. All the sampled plants affected by leaf loss maintained some green leaves, vital buds, and green bark under the periderm and were considered to be alive in most cases, as only 1.7% of the plants in the "leaf loss" category appeared as dead (without the above-mentioned signs) at the end of the

study.

Half the plants from each canopy dieback category were randomly sampled in April 2012, at the peak of the drought period (hereafter, drought), while the other half were sampled in February 2013, after the autumn rain pulse (hereafter, post-rain) (Fig. 1C), just before the start of the new growing season. Our study does not focus on phenological NSC dynamics, but rather on its variations in water availability during an extreme drought episode and after subsequent rainfall. At each sampling time, leaf loss levels were visually estimated as a percentage for each individual plant in relation to the undefoliated individuals found in the study area, as in Sapes *et al.*, (2017).

### ***Functional trait measures***

Study species were sampled to collect information about several functional traits: (1) wood density (WD), which is related to conductive efficiency, as well as to growth potential and resistance to several stress and disturbance agents (Chave *et al.*, 2009; Pérez-Harguindeguy *et al.*, 2013); (2) plant height ( $H_{\max}$ ), which is related to growth form, competitive vigour, and potential lifespan, and (3) specific leaf area (SLA, leaf area per unit of dry leaf mass), which is related to resource acquisition and leaf turnover (Wright *et al.*, 2004). Functional trait data were obtained from six representative individuals with no signs of canopy dieback per species of the studied community in May 2014. All trait measurements were conducted following standardized protocols (Pérez-Harguindeguy *et al.*, 2013). WD ( $\text{mg mm}^{-3}$ ) was measured after removing the bark from 10-cm long stem segments from each plant. In the

case of *P. halepensis*, we used cores obtained with tree-ring borers. We used the water displacement method to determine fresh volume and we dried all the samples in an oven at 70 °C for 72h until a constant weight was obtained. Plant height (cm) was measured as the shortest distance between the upper limit of the main photosynthetic tissues (excluding inflorescences) and the ground level, and  $H_{\max}$  was determined as the maximum of these values for a given species. SLA ( $\text{cm}^2 \text{g}^{-1}$ ) was estimated for each individual as the mean value of 20 fully expanded, undamaged sun leaves from the current year. Fresh leaves were digitally scanned and analyzed with Image-J software (from the US National Institute of Health; <http://www.nih.gov/>, accessed 22 February 2013). Leaves were then oven-dried at 70 °C for at least 72h and weighed to the nearest 0.0001 g. SLA was calculated as the ratio between the area of the leaf lamina and its dry mass.

#### ***Carbohydrate sampling and analyses***

For each plant, we collected stem samples that were located within the first ten centimetres above ground surface. Similarly, root samples were collected within the ten first centimetres below ground surface. Root and stem samples were transported in a cooler over ice until sample processing. On the same day of collection, samples were microwaved for 90 s to stop enzymatic activity and subsequently oven-dried for 72 h at 65 °C. Heartwood and inner bark were removed from the sample before grinding into fine powder for the non-structural carbohydrate (NSC) analyses. NSCs were defined as low molecular weight soluble sugars (SS) (glucose and fructose, sucrose, and other free sugars) plus starch, and they were analyzed following the procedures described by Hoch *et al.*, (2002) and Galiano *et al.*, (2012). Sapwood powder (~12–14 mg) was extracted with 1.6 ml distilled water at 100 °C for 60 min. After centrifugation, an aliquot of the extract was used to determine SS content after

the enzymatic conversion of sucrose and fructose into glucose (using invertase from *Saccharomyces cerevisiae* and glucose hexokinase (GHK) assay reagent, I4504 and G3293, Sigma-Aldrich, Spain). NSC content was obtained from another aliquot that was incubated in amyloglucosidase from *Aspergillus niger* (10115 Sigma-Aldrich) at 50 °C overnight, to break down all NSCs (starch included) to glucose. The concentration of free glucose was determined photometrically in a 96-well microplate reader (Sunrise™ Basic Tecan, Männedorf, Switzerland) after enzymatic (GHK assay reagent) conversion of glucose into gluconate-6-phosphate. Then, the dehydrogenation of glucose causes an increase in optical density at 340 nm. All the NSC and SS contents were expressed as percent dry matter (%DM).

We computed several indices at the species level to describe the proportion of NSC and SS in the different organs, canopy dieback states, and periods. We calculated the ratio of NSC and SS in roots relative to stems (considering the values of all plants, regardless of leaf loss) in both the drought and post-rain periods; this ratio indicates the differences in C allocation between organs in the various species. We also calculated the ratio of NSC and SS between undefoliated plants and those with leaf loss (by averaging stems and root values) for both periods; this ratio indicates differences associated with canopy dieback in the various species. Finally, we calculated the species' difference and relative change (value in the post-rain period minus value in the drought period / value in the drought period) in NSC and SS (by averaging the stem and root values of all the plants, regardless of leaf loss) and green canopy (again by averaging all the plants) between the two studied periods.

## ***Statistical analyses***

We tested the existence of differences in NSC and SS concentrations between roots and stems, between species, and between green and defoliated plants using General Linear Mixed Models (GLMMs). Separate models were built for samples collected in April 2012 (drought) and in February 2013 (post-rain). Both models included either NSC (log-transformed) or SS concentration (log-transformed) as the dependent variable and organ (root, stem), canopy dieback status (undefoliated, leaf loss), and species as fixed factors. Two-way interactions between fixed factors were included in the model to test whether canopy dieback status influenced NSC and SS contents differentially between organs and species, and whether differences in NSC and SS concentrations at the organ level were species-specific. Plant identity was also included in the models as a random factor to account for plant effects on NSC concentration in the different organs of a single individual. Post-rain models also included percentage of green canopy, since we assumed that this percentage largely reflected autumn rain and could explain NSC concentrations after rain. Samples in which chemical analyses failed to obtain photometrical readings above zero (probably due to low NSC concentrations) were excluded from data analyses.

We tested for differences in NSC and SS concentration between drought and post-rain periods using GLMMs. The models included NSC and SS (log-transformed) as dependent variables; sampling time (drought, post-rain), organ, canopy dieback status during the drought period, and species as fixed factors; and plant identity as a random factor. Two-way interactions were included in the models, for the reasons explained above. We conducted within-species comparisons of the main factors of the different models for each sampling date and between sampling dates using least square means difference (LSMD) Student's t-tests.

We also tested for relationships between the recovery of species' green canopy between the two periods (difference in green foliage) and both NSC and SS concentration in the drought period and changes in NSC and SS between the two periods, using Spearman's non-parametric correlations. Relationships between species' functional traits (log-transformed) and NSC and SS concentrations (averaging the stem and root values of all plants) during drought and post-rain periods were explored using Phylogenetic Generalized Least Squares (PGLS). We separately analyzed each variable describing species' functional traits since no significant correlation was observed between them. We also used PGLS to relate functional traits to (1) the proportion of NSC and SS existing in roots relative to stems; (2) the ratio of NSC and SS between undefoliated plants and those with leaf loss; (3) the species' difference and relative change in NSC and SS between the two studied periods, determined as explained above.

Statistical analyses were performed with JMP10.0 (SAS Institute Inc.), except for PGLS analyses, which were performed with R software (version 3.1, R Foundation for Statistical Computing, Vienna, AT) using *phylotools* (Zhang *et al.*, 2012), *ape* (Paradis *et al.*, 2004), and *caper* (Orme *et al.*, 2013) packages. Phylogenetic relatedness between species was estimated with Phylomatic (Webb and Donoghue, 2004) and Phylocom (Webb *et al.*, 2008).

## **Results**

### ***Carbohydrates during the drought period***

Plants with leaf loss exhibited a significantly lower NSC concentration (15.7%) compared to undefoliated plants (averaging the stem and root values) (Figure 2) [Supplementary



Information – Table 1]. This effect was consistent in both stems and roots. The species in which these differences were largest were *Q. coccifera* (LSMD t ratio=2.59,  $P=0.010$ ), *R. lycioides* (LSMD t ratio=2.90,  $P=0.029$ ), *G. scorpius* (LSMD t ratio=3.02,  $P=0.003$ ), and *R. officinalis* (LSMD t ratio=4.70,  $P<0.001$ ) (Table 2) [Supplementary Information – Table 1]. In *Q. coccifera* low NSC were mostly due to low root concentration in plants with leaf loss, while in *G. scorpius* they were due to stems. In *R. lycioides* and *R. officinalis* the differences between plants with leaf loss and undefoliated ones were not significant when the organs were considered separately.

In contrast to NSC, the overall effect of leaf loss on SS was only marginally significant. In plants with leaf loss SS decreased by only 5.9%, compared to undefoliated plants (averaging the stem and root values) (Figure 2) [Supplementary Information – Table 1]. The differences were only significant in *R. lycioides* (LSMD t ratio=2.85,  $P=0.005$ ) and *R. officinalis* (LSMD t ratio=4.18,  $P<0.001$ ), mostly due to lower SS values in the roots of plants with leaf loss (Table 2) [Supplementary Information – Figure 1].

Overall, NSC concentration was 1.4 times higher in roots than in stems (Table 2, Figure 2) [Supplementary Information – Table 1]. The exceptions were *L. fruticosum* and *T. vulgaris*, where NSC concentration did not differ in the roots and stems, and in *R. officinalis*, where the values were higher in stems (LSMD t ratio=4.23,  $P<0.001$ ) [Supplementary Information – Figure 1].

The overall SS concentration did not vary significantly between organs. The exceptions were *R. lycioides* and *R. officinalis*, which had a lower SS content in roots than in stems (LSMD t ratio=2.28,  $P=0.024$  and LSMD t ratio=3.50,  $P<0.001$ , respectively), and *T. tinctoria*, which

exhibited the opposite pattern (LSMD t ratio=2.25,  $P=0.020$ ) (Table 2) [Supplementary Information – Figure 1].

### ***Carbohydrates and green foliage in the post-rain period***

After the rain, plants with leaf loss in the drought period did not differ significantly in NSC concentration from undefoliated ones (Table 2; Figure 2). In contrast, the SS concentration was higher overall in plants that exhibited leaf loss, compared to undefoliated ones (Table 2, Figure 2), particularly in stems of *J. phoenicea* (LSMD t ratio=2.91,  $P=0.002$ ) and roots of *Q. coccifera* (LSMD t ratio=2.09,  $P=0.019$ ) [Supplementary Information – Figure 1].

As during the drought period, the overall NSC concentration was 1.4 times higher in roots relative to stems (averaging all the plants) but this pattern varied strongly between species: in *T. vulgaris*, the roots had lower NSC than the stems (LSMD t ratio=4.33,  $P<0.001$ ) but the differences were not significant in *Q. coccifera*, *L. fruticosum*, or *R. officinalis* (Table 2) [Supplementary Information – Figure 1].

Overall, SS concentration did not vary significantly between organs, but some species did present different patterns. The SS concentration was lower in roots, compared to stems, in *J. phoenicea* (LSMD t ratio=2.14,  $P=0.034$ ) and *Quercus coccifera* (LSMD t ratio=2.21,  $P=0.029$ ), but the reverse was true in *P. halepensis* (LSMD t ratio=3.41,  $P<0.001$ ) and *T. tinctoria* (LSMD t ratio=3.36,  $P=0.001$ ) (Table 2) [Supplementary Information – Figure 1].

In the post-rain sampling, the percentage of green canopy had a stronger positive effect on both NSC and SS concentrations than canopy dieback status (Table 2).

### ***Comparison between periods***

Green canopy increased more in plants with leaf loss. This recovery was particularly strong in *P. halepensis*, *L. fruticosum*, *R. officinalis*, and *T. vulgaris*. In contrast, *G. scorpius* showed a clear decrease in the green foliage of undefoliated plants (Figure 3).

Overall, the NSC concentration decreased from the drought to the post-rain period (Table 3) [Supplementary Information – Table 1]. Generally speaking, this decrease was consistent between organs, canopy dieback status during the drought, and species (Table 3), in spite of some discrepancies in specific species [Supplementary Information – Figure 1]. Exceptions to this overall pattern were found, for example, in *R. officinalis*, where root NSC increased in plants with leaf loss (LSMD t ratio=5.47,  $P<0.001$ ), and in *P. halepensis*, where NSC decreased in stems (LSMD t ratio=2.19,  $P=0.014$ ) but not in roots (LSMD t ratio=0.32,  $P=0.745$ ).

In contrast to NSC, SS concentration generally increased in the post-rain period [Supplementary Information – Table 1]. This trend occurred in both organs and plants with different canopy dieback status in the drought period. However, SS increase varied between species (Table 3): it was significant in *Q. coccifera* (LSMD t ratio=5.82,  $P<0.001$ ), *R. lycioides* (LSMD t ratio=4.23,  $P<0.001$ ), *G. scorpius* (LSMD t ratio=2.89,  $P=0.004$ ), and *L.*

*fruticosum* (LSMD t ratio=2.95,  $P=0.003$ ) [Supplementary Information – Figure 1]. In *R. officinalis*, SS increased in plants with leaf loss (LSMD t ratio=3.13,  $P=0.002$ ) but not in undefoliated ones (LSMD t ratio=0.39,  $P=0.700$ ).

The recovery of green canopy in plants with leaf loss was negatively correlated with root NSC concentration in the drought period across the species (Spearman  $\rho = -0.882$ ,  $P=0.002$ ) (Figure 4A). This pattern was also observed in the stems (Spearman  $\rho = -0.683$ ,  $P=0.042$ ) and roots (Spearman  $\rho = -0.683$ ,  $P=0.042$ ) of undefoliated plants. The recovery of species' green canopy was not significantly related to SS concentration in the drought period or to the change in either NSC or SS between periods.

#### ***Relationships between traits and carbohydrate content***

WD was related to some aspects of C dynamics. During the drought period, low WD species (particularly *T. tinctoria*, *L. fruticosum*, *R. officinalis*, and *T. vulgaris*) had a significantly higher fraction of NSC composed of SS, compared to high WD species (*P. halepensis*, *J. phoenicea*, *Q. coccifera*, *R. lycioides* and *G. scorpius*; PGLS,  $\lambda=0.993$ ,  $t=2.45$ ,  $P=0.044$ ; Fig. 5A). This relationship exhibited a phylogenetic signal, probably associated with the different behaviour of the gymnosperm *P. halepensis*. Similarly, higher WD was associated with greater relative increases in SS from the drought to the post-rain periods, irrespective of lineage effect (PGLS,  $\lambda=0$ ,  $t=3.04$ ,  $P=0.023$ ) (Fig. 5B). Finally, during the post-rain period lower WD species exhibited greater depletion of NSC in plants with leaf loss, compared to undefoliated ones,

irrespective of lineage effect (PGLS,  $\lambda=0$ ,  $t=2.40$ ,  $P=0.047$ ) (Fig. 5C). No other significant relationship was found between WD and NSC or green canopy change.

Species'  $H_{\max}$  and SLA were not statistically related to C concentration and dynamics or to canopy recovery. However, small species tended to have higher concentrations of SS in undefoliated plants than in plants with leaf loss after the rain period (negative relationship between  $H_{\max}$  and undefoliated plants to plants with leaf loss SS ratio, PGLS,  $\lambda=1$ ,  $t=2.28$ ,  $P=0.057$ ) and higher NSC concentration in stems than in roots (positive relationship between  $H_{\max}$  and root to stem ratio, PGLS,  $\lambda=1$ ,  $t=2.32$ ,  $P=0.054$ ).

## Discussion

Our community-level study revealed that NSC as a whole and the SS fraction responded differently to a sequence of intense drought followed by rain. There was a decrease in the overall NSC stocks in shrubs with symptoms of canopy dieback due to prolonged drought, compared to those of undefoliated shrubs. On average, NSC stocks continued to decrease after the rain, likely because canopy recovery incurs initial carbon costs. In contrast to NSC, SS concentrations were similar between plants with and without leaf loss during the drought, and they increased after the rain event. Such SS dynamics are consistent with the critical role of SS in metabolism and water relationships during drought (O'Brien *et al.*, 2014; Sevanto *et al.*, 2014; García-Forner *et al.*, 2016). Despite these general trends, coexisting species presented different C dynamics, which were in part related to wood density, a trait related to growth rates and long-distance water transport.

## *Carbohydrate dynamics*

As hypothesized, plants exhibiting foliage loss (canopy dieback) during the drought (with foliage loss) had lower NSC concentrations in both stems and roots than undefoliated plants, as previously observed in the roots of other Mediterranean trees under water stress (Klein *et al.*, 2014). This decrease occurred in both stems and roots. Roots were the main NSC storage organ, as expected in Mediterranean systems where above-ground disturbances are common (Martínez-Vilalta *et al.*, 2016). NSC depletion was similar in both organs, suggesting that C demand or mobilization was also similar in above- and below-ground organs. These lower NSC concentrations in plants with leaf loss during drought occurred despite potential NSC accumulation that can occur when drought decreases growth more than photosynthesis (Körner, 2003).

NSC concentration in most species did not increase after the autumn rain period. On the contrary, on average, NSC decreased regardless of the degree of canopy dieback at the peak of the drought. This suggests that newly assimilated carbon after seasonal pulses of rain was insufficient to meet carbon demand for new tissue growth after a prolonged drought. The low NSC concentrations found in the post-rain period (February) before the start of the new growing season could also reflect temperature limitations on wintertime carbon assimilation (Terradas, 1986; Camarero *et al.*, 2010). Temperature-limited carbon assimilation is relatively common in some (De Lillis and Fontanella, 1992; Llorens *et al.*, 2003; Asensio *et al.*, 2007; Vaz *et al.*, 2010), though not all (Körner, 2003) Mediterranean-type ecosystems.

1 The significant increase in SS after the autumn rain does suggest a general increase in  
2 physiological activity, as sugars were mobilized for growth and metabolic demands. An  
3 autumnal increase in physiological activity occurs in Mediterranean-type ecosystems with  
4 strong seasonal trends in precipitation, resulting in peaks of assimilation in both spring and  
5 autumn, due to the high rainfall and mild temperatures (Larcher, 2000; Llorens *et al.*, 2003).  
6 The seasonality of Mediterranean-type ecosystems is essential to any interpretation of our  
7 results, which were probably influenced by both the specific conditions of the year of the  
8 study (within a multi-year drought period), and the timing of the second sampling, after a  
9 peak of autumn activity and before a second peak of potential productivity in spring.

10  
11 The observed values of SS in plants with leaf loss agree with experimental evidence  
12 suggesting that NSC storage mitigates the effects of drought (Woodruff and Meinzer, 2011;  
13 O'Brien *et al.*, 2014). One consequence of drought is turgor loss (Bartlett *et al.* 2012), along  
14 with potential loss of water transport, desiccation and death (Tyree *et al.* 2003). A main  
15 mechanism by which cells maintain turgor under drought is via the accumulation of  
16 osmotically active compounds such as SS (Sala and Mencuccini, 2014; O'Brien *et al.*, 2014;  
17 Sevanto *et al.*, 2014), which are converted from starch (McDowell *et al.*, 2011; García-Forner  
18 *et al.*, 2016). Furthermore, starch-derived SS might also play a critical role in xylem repair  
19 and vascular transport (De Baerdemaeker *et al.*, 2017). Our results are consistent with this  
20 interpretation because, compared to NSC, SS remained relatively stable across species when  
21 plants with leaf loss were compared with their undefoliated counterparts, even though SS is a  
22 source of C for respiration (cf. Hartmann and Trumbore, 2016; Martínez-Vilalta *et al.*, 2016).  
23 Recent pulse-labelling experiments further suggest that SS may be actively accumulated  
24 during water shortage and subsequent recovery at the expense of short-term growth,  
25 presumably to optimize growth and survival in the long term (Hartmann *et al.*, 2015; Galiano

1 *et al.*, 2017). In our case, such accumulation appeared to be species- and organ-specific. For  
2 instance, in *R. lycioides* and *R. officinalis*, root SS were lower during the drought period than  
3 after the rain.

4  
5 SS concentration increased after the rain pulse, which was not the case for total NSC. This  
6 increase is expected when physiological activity resumes and sugars are used for growth and  
7 metabolic demands, and may be due to: i) temporary increases in assimilation rates and/or  
8 reductions in carbon demand or ii) the conversion of starch into SS. In the first case, these  
9 adjustments would yield a net carbon gain, which would be observed first as SS, given that  
10 SS are both the direct product of assimilation and the substrate of most anabolic reactions. In  
11 the second case, a conversion of starch from stems and roots into SS would yield higher SS  
12 concentrations without any increases in total NSC. Our results are consistent with this second  
13 interpretation, as NSC either declined or remained constant in nearly all the species in our  
14 study.

### 16 ***Recovery of green foliage***

17  
18 We hypothesized that the recovery of green foliage after the autumn rainy period would  
19 consume stored NSC and would thus result in lowered NSC concentrations in both stems and  
20 roots. Consistent with this, the NSC average across species did show lower concentrations  
21 after the drought period, compared to those found during drought (Figure 2), coinciding with  
22 an increase in green foliage in most species (Figure 3). Therefore, the decrease in NSC from  
23 the drought to the post-rain period appeared to correspond to growth-related carbon demands.  
24 Previous studies have documented reductions in NSC concentrations associated with the  
25 production of new foliage after drought (Bréda *et al.*, 2006; Galiano *et al.*, 2011; 2012) or



1 other disturbances (e.g., Canadell and López Soria, 1998; Palacio *et al.*, 2012). NSC  
2 concentrations were particularly low in plants with leaf loss, probably because they grew  
3 more leaves in the autumn. There was no relationship, however, between the drop in NSC  
4 from drought to post-rain and the canopy recovery across species (i.e., species with a higher  
5 canopy recovery did not show stronger decreases in NSC), probably because the balance  
6 between assimilation and growth or other demands varied across species.

7

8 Interestingly, foliage recovery tended to correlate negatively with NSC concentration during  
9 the drought. This could occur if species that approached their lowest NSC thresholds during  
10 drought subsequently maximized the production of new leaves. This response would  
11 minimize the risk of complete depletion of reserves in the mid-term (Martínez-Vilalta *et al.*,  
12 2016). In fact, plants with leaf loss did not show greater reductions in NSC between the two  
13 study periods, despite the fact that these plants had less NSC at the peak of the drought and  
14 recovered more green foliage on average (Figure 4). That is, plants that experienced leaf loss  
15 appeared to minimize NSC depletion during canopy recovery perhaps due to greater (or  
16 faster) returns from new leaves. Variability between species, however, was substantial. In  
17 general, NSC continued to decrease after the drought, concurrent with a lack of canopy  
18 recovery; see *P. halepensis*, *Q. coccifera* *G. scorpius*, and *T. vulgaris* [Supplementary  
19 Information – Figure 1], perhaps reflecting a legacy of drought effects. In *R. officinalis*,  
20 which had severe leaf loss during the drought, root NSC increased during canopy recovery,  
21 consistent with reports of a fast replenishment of carbon stores after disturbance (e.g., Palacio  
22 *et al.*, 2012). Our study shows that NSC use for rebuilding canopies generally exceeded any  
23 new formation of reserves derived from relatively high rates of assimilation in autumn.

24 Therefore, despite occasional rain events, if drought persists in the long term, plants may fall  
25 into a feedback loop in which storage is eventually insufficient to grow new leaves, in turn

reducing canopy assimilation and NSC formation. Such dynamics could ultimately compromise the capacity to resist frequent droughts (Galiano *et al.*, 2011).

### ***Relationships between traits and carbohydrate content***

We did not find any clear association between acquisitive leaf traits (high SLA) or plant longevity (high  $H_{max}$ ) and higher NSC variability, as we had hypothesized. Only WD could explain, to some extent, differences in C dynamics across species. Low WD is generally correlated with high growth rates (Poorter *et al.*, 2008; Greenwood *et al.*, 2017), low resistance to xylem embolism, but more efficient water transport relative to denser wood which is more mechanically stable (Baas *et al.*, 2004; Chave *et al.*, 2009). The greater proportion of SS relative to total NSC in low-WD species relative to high-WD species may therefore reflect their overall greater metabolic activity. Consistently, most of the small trees and large shrubs in the community (i.e. largest but presumably slower growing plants) had high WD and a low proportion of SS relative to the total NSC (Fig. 5a). An exception was *P. halepensis*, a fast-growing gymnosperm tree which despite its low WD, exhibited low SS concentration compared to the total NSC. However, relative to angiosperms, gymnosperms have much less parenchyma tissue, and consequently lower C storage capacity and subsequent SS depolymerization (Johnson *et al.*, 2012; Morris *et al.*, 2016). Within low-WD species, we also found that during the drought NSC was similar between plants with and without leaf loss, but after the rain plants with leaf loss exhibited lower NSC than undefoliated plants. The generally faster growth of lower WD species may predispose those that become defoliated under drought to consume more carbon for foliage recovery after the autumn rain.

1 Patterns of growth and related C dynamics were expected to differ in the contrasting life  
2 forms considered in our study (from suffrutescent small shrubs to trees). Plant size – measured  
3 in our study by  $H_{\max}$  – constitutes one of the two major axes explaining trait variability at a  
4 global scale (Díaz *et al.*, 2016), and it is often associated with faster growth rates (Díaz *et al.*,  
5 2016), higher longevity, and higher competitive ability due to the pre-emption of light  
6 resources. In our community, shorter species, (*T. tinctoria*, *L. fruticosum*, *T. vulgaris*) had a  
7 shorter lifespan and lower long-term competitive ability. We failed to find any clear  
8 relationship between  $H_{\max}$  and C dynamics, probably due to the low number of studied  
9 species and the multiple functions associated with this single trait. Nevertheless, we observed  
10 a negative but weak relationship between  $H_{\max}$  and concentration of SS in undefoliated plants  
11 after the rain period, which is consistent with a faster response to pulses of resources in  
12 smaller, short-lived species.

13  
14 SLA indicates the carbon cost to replace the canopy after leaf loss. It is also positively related  
15 to growth rates and photosynthetic potential (Poorter *et al.*, 2009). However, high SLA has  
16 been recently related with susceptibility to drought-induced mortality (Greenwood *et al.*,  
17 2017). However, we found no relationship between SLA and NSC or SS dynamics. This is  
18 likely because some species in arid ecosystems often use an avoidance-type strategy whereby  
19 rapid leaf turnover allows the regulation of leaf area and water loss (Mooney and Dunn,  
20 1970). This rapid leaf turnover in drought-avoiders often places these species at the low-cost,  
21 rapid-investment-return end of the leaf-economy spectrum. Thus, plant communities in arid  
22 ecosystems tend to be composed of a mixture of drought-avoidant (i.e., high SLA) and  
23 drought-tolerant (i.e., low SLA) species, which makes the correlations between leaf-economy  
24 traits and climate rather weak. In fact, Wright *et al.* (2004) found a strong relationship  
25 between leaf mass per area (the inverse of SLA) and climate in evergreen species but not in

deciduous species, including summer deciduous species. Summer deciduousness is common in Mediterranean communities like the one that we studied (Zunzunegui *et al.*, 2005). In our case, fast foliage recovery in several potentially drought-avoidant small species (*T. vulgaris*, *L. fruticosum*) (see also Lloret *et al.*, 2016) may have blurred any relationship between SLA and NSC dynamics. Furthermore, the relationships between traits (including SLA) and demographic rates often depend on the ontogenetic state and can also be related to plant size (Gibert *et al.*, 2016). This limits inferences, particularly when considering small sets of species with a wide range of growth forms and phylogenies, as in our study.

### ***Concluding remarks***

Our study showed a decrease of NSC stocks in woody plants with canopy dieback in response to a prolonged drought. Subsequent growth stimulation after stochastic pulses of water availability coinciding with mild temperatures incurred further carbon costs. These results indicate that recovery of NSC reserves would require subsequent periods of average or above-average precipitation in the mid-term. Persistent long-term drought, even when interrupted by occasional rain pulses, could eventually deplete NSC stocks to the point that canopy recovery is no longer possible. Such an effect is likely to occur in many ecosystems predicted to experience increasing aridity and climatic variability in the future where short-term canopy recovery and subsequent carbon assimilation after rain events may not ensure long-term resilience. Because changes in plants' C dynamics may be difficult to detect via integrative measures obtained at the stand or landscape levels, such as remote sensing imagery, the assessment of vegetation conditions over extensive temporal or spatial scales remains challenging.

1 Our study also shows substantial species-specific variability in C dynamics and highlights the  
2 importance of taking into account phenological effects. Additional research is therefore  
3 required to fully understand species-specific carbon dynamics under drought in  
4 Mediterranean species and their consequences on resilience to drought. Nevertheless, the  
5 variable responses we found in coexisting species suggests a range of adaptive abilities that  
6 could promote coexistence in a changing climate. Recent studies have indeed highlighted the  
7 role of functional diversity in enhancing resilience to extreme climatic episodes (de la Riva *et*  
8 *al.*, 2017). Plant functional traits are increasingly used as a tool to compare species'  
9 functional responses and to upscale to community and ecosystem levels, where  
10 environmental gradients are more conspicuous. In our study, however, only one specific trait  
11 (WD) was related to ecophysiological responses (C economy) to particular climatic events,  
12 highlighting the importance of selecting appropriate traits and the difficulty in making  
13 generalizations based on the relationships between sets of traits (e.g., acquisitive vs.  
14 conservative classifications) and plants' responses to climatic disturbance.

**Supplementary Information Table 1.** Mean non-structural carbohydrates (NSC) and soluble sugar (SS) concentrations in stems and roots of undefoliated and plants with leaf loss from the set of species during the drought and post-rain periods.

**Supplementary Information Figure 1.** Non-structural carbohydrates (NSC) and soluble sugar (SS) concentrations of the different species in stems and roots of undefoliated plants and those with leaf loss (dieback) during the drought (April 2012) and post-rain (February 2013) periods.

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## REFERENCES

- Adams HA, Zeppel MJB, Anderegg WRL, *et al.* 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology and Evolution* **1** : 1285–1291.
- Aguadé D, Poyatos R, Gómez M, Oliva J, Martínez-Vilalta J. 2015. The role of defoliation and root rot pathogen infection in driving the mode of drought-related physiological decline in Scots pine (*Pinus sylvestris* L.). *Tree Physiology* **35**: 229–242.
- Allen CD, Macalady AK, Chenchouni H *et al.* 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology Management* **259** : 660–684.
- Anderegg, W. R. L., Berry, J.A., Smith, D.D., Sperry, J. S., Anderegg, L. D. L., Field, C. B. 2012. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings National Academy Sciences U. S. A.* **109** : 233–237.
- Asensio D, Peñuelas J, Ogaya R, Llusia J. 2007. Seasonal soil and leaf CO<sub>2</sub> exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. *Atmospheric Environment* **41** : 2447-2455.
- Baas P, Ewers FW, Davis SD, Wheeler EA. 2004. Evolution of xylem physiology. In: Hemsley A, Poole I, eds. *The Evolution of Plant Physiology*, Academic Press, 273–295.

- Bartlett MK, Scoffoni C, Sack, L. 2012.** The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. *Ecology Letters* **15** : 393–405.
- Boehmer HJ, Wagner HH, Jacobi JD, Gerrish GC, Mueller-Dombois D. 2013.** Rebuilding after collapse: Evidence for long-term cohort dynamics in the native Hawaiian rain forest. *Journal Vegetation Science* **24** : 639–650.
- Bolòs O, Vigo, J, Masalles RM, Ninot JM. 2005.** *Flora manual dels Països Catalans*, 3rd edn. Barcelona: Pòrtic.
- Braun-Blanquet J, Bolòs O. 1957.** The plant communities of the Central Ebro Basin and their dynamics. *Anales Estación Experimental Aula Dei* **5** : 1–266.
- 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. *Annals Forest Science* **63** : 625-644.
- Breshears, D. D., Cobb NS, Rich PM, et al. 2005.** Regional vegetation die-off in response to global-change-type drought. *Proceedings National Academy Sciences U. S. A.* **102** : 15144–15148).
- Briffa KR., Van Der Schrier G, Jones PD. 2009.** Wet and dry summers in Europe since 1750. *International Journal Meteorology* **29** : 1894-1905.



- 1 **Camarero JJ, Olano JM, Paras A. 2010.** Plastic bimodal xylogenesis in conifers from  
2 continental Mediterranean climates. *New Phytologist* **185** : 471-480.
- 3
- 4 **Canadell J, López-Soria L. 1998.** Lignotuber reserves support regrowth following clipping  
5 of two Mediterranean shrubs. *Functional Ecology* **12** : 31-38.
- 6
- 7 **Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J. 2011.** Widespread  
8 crown condition decline, food web disruption, and amplified tree mortality with increased  
9 climate change-type drought. *Proc Natl Acad Sci U S A.* 108: 1474–8.  
10 doi:10.1073/pnas.1010070108
- 11
- 12 **Chapin FS, Schulze E-D, Mooney HA. 1990.** The ecology and economics of storage in  
13 plants. *Annual Review of Ecology and Systematics* 21:423–447.
- 14
- 15 **Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009.** Towards a  
16 worldwide wood economics spectrum *Ecology Letters* **12** : 351-366.
- 17
- 18 **Dai, A. 2012.** Increasing drought under global warming in observations and models. *Nature*  
19 *Climate Change* **3** : 52-58.
- 20
- 21 **De Baerdemaeker NJF, Salomon RL, De Roo L, Steppe K. 2017.** Sugars from woody  
22 tissue photosynthesis reduce xylem vulnerability to cavitation. *New Phytologist* **216** : 720-  
23 727.
- 24
- 25

- 1 **De la Riva E, Lloret F, Pérez-Ramos I et al. 2017.** The importance of functional diversity  
2 in the stability of Mediterranean shrubland communities after the impact of extreme climatic  
3 events. *Journal of Plant Ecology* **10** : 281-293.
- 4
- 5 **De Lillis M, Fontanella A. 1992.** Comparative phenology and growth in different species of  
6 the Mediterranean maquis of central Italy. *Vegetatio* **100** : 83-96.
- 7
- 8 **Díaz S, Kattge J, Cornelissen JHC et al. 2016.** The global spectrum of plant form and  
9 function. *Nature* **529** : 167–171.
- 10
- 11 **Dobbertin M, Brang P. 2001.** Crown defoliation improves tree mortality models. *Forest*  
12 *Ecology Management* **141** : 271–284.
- 13
- 14 **Galiano L, Martínez-Vilalta J, Lloret F. 2011.** Carbon reserves and canopy defoliation  
15 determine the recovery of Scots pine 4yr after a drought episode. *New Phytologist* **190** : 750–  
16 759.
- 17
- 18 **Galiano L, Martinez-Vilalta J, Sabate S, Lloret F. 2012.** Determinants of drought effects  
19 on crown condition and their relationship with depletion of carbon reserves in a  
20 Mediterranean holm oak forest. *Tree Physiology* **32** : 478–489.
- 21
- 22 **Galiano L, Timofeeva G, Saurer M et al. 2017.** The fate of recently fixed carbon after  
23 drought release: towards unravelling C storage regulation in *Tilia platyphyllos* and *Pinus*  
24 *sylvestris*. *Plant Cell Environment* **40** : 1711-1724.
- 25

1 **Gao XJ, Pal JS, Giorgi F. 2006.** Projected changes in mean and extreme precipitation over  
2 the Mediterranean región from a high resolution double nested RCM simulation. *Geophysical*  
3 *Research Letters* **33** : Article Number: L03706. doi: 10.1029/2005GL024954.

4  
5 **García-Forner N, Sala A, Biel C *et al.* 2016.** Individual traits as determinants of time to  
6 death under extreme drought in *Pinus sylvestris* L. *Tree Physiology* **36** : 1196-1209.

7  
8 **Giannakopoulos C, Le Sage P, Bindi M, Moriondo M, Kostopoulou E, Goodess CM.**  
9 **2009.** Climatic changes and associated impacts in the Mediterranean resulting from a 2 °C  
10 global warming. *Global Planetary Change* **68** : 209-224.

11  
12 **Gibert A, Gray EF, Westoby M, Wright IJ, Falster DS. 2016.** On the link between  
13 functional traits and growth rate: meta-analysis shows effects change with plant size, as  
14 predicted. *Journal of Ecology* **104** : 1488–1503.

15  
16 **Greenwood S, Ruiz-Benito P, Martínez-Vilalta J *et al.* 2017.** Tree mortality across biomes  
17 is promoted by drought intensity, lower wood density and higher specific leaf area. *Ecology*  
18 *Letters* , in press. doi:10.1111/ele.12748.

19  
20 **Gruber A, Pirkebner D, Florian C, Oberhuber W. 2012.** No evidence for depletion of  
21 carbohydrate pools in Scots pine (*Pinus sylvestris* L.) under drought stress. *Plant Biology* **14** :  
22 142–148.

23

**Hacke UG, Sperry JS, Pockman WT, Davis SD, Mcculloh KA. 2001.** Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126** : 457–461.

**Hagedorn F, Joseph J, Peter M *et al.* 2016.** Recovery of trees from drought depends on belowground sink control. *Nature Plants* **2** : art 16111. doi:10.1038/nplants.2016.111.

**Hartmann H, Ziegler W, Kolle O, Trumbore S. 2013.** Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. *New Phytologist* **200** : 340–349.

**Hartmann H, McDowell NG, Trumbore S. 2015.** Allocation to carbon storage pools in Norway spruce saplings under drought and low CO<sub>2</sub>. *Tree Physiology* **35** : 243–252.

**Hartmann H, Trumbore S. 2016.** Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. *New Phytologist* **211** : 386–403.

**Hoch G, Popp M, Körner C. 2002.** Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* **98** : 361–374.

**Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC. 2012.** Hydraulic safety margins and embolism reversal in stems and leaves: Why are conifers and angiosperms so different? *Plant Science* **195** : 48–53.

**Jump AS, Ruiz-Benito P, Greenwood S, Allen CD, Kitzberger T, Fensham R, Martínez-Vilalta J, Lloret F. 2017.** Structural overshoot of tree growth with climate variability and the global spectrum of drought-induced forest dieback. *Global Change Biology*, **23** : 3742-3757.

**Klein T, Hoch G, Yakir D, Körner C. 2014.** Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest. *Tree Physiology* **34** : 981-992.

**Körner C. 2003.** Carbon limitation in trees. *Journal of Ecology* **91** : 4–17.

**Larcher W. 2000.** Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosystems* **134** : 279-295.

**Llorens L, Peñuelas J, Filella I. 2003.** Diurnal and seasonal variations in the photosynthetic performance and water relations in two co-occurring in two Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*. *Physiologia Plantarum* **118** : 84-95.

**Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F. 2012.** Extreme climatic events and vegetation: the role of stabilizing processes. *Global Change Biology* **18** : 797-805.

**Lloret F, de la Riva E, Pérez-Ramos I et al. 2016.** Climatic events inducing die-off in Mediterranean shrublands: Are species responses related to their functional traits? *Oecologia* **180** : 961–973.

1

2 **Martinez-Vilalta J, Sala A, Asensio MD, et al. 2016.** Dynamics of non-structural

3 carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs* **86** : 495-516.

4

5 **McDowell NG, WT Pockman, Allen CD et al. 2008.** Mechanisms of plant survival and

6 mortality during drought: why do some plants survive while others succumb to drought? *New*

7 *Phytologist* **178** :719–739.

8

9 **McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M. 2011.** The

10 interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in*

11 *Ecology and Evolution* **26** : 523–532.

12

13 **McDowell NG, Fisher R, Xu C et al. 2013.** Uncertainties and opportunities in modeling

14 drought-associated vegetation mortality. *New Phytologist* **200** : 304–321.

15

16 **McDowell, NG, Sevanto S. 2010.** The mechanisms of carbon starvation: how, when, or does

17 it even occur at all? *New Phytologist* **186** : 264-266

18

19 **McKell CM. 1975.** Shrubs – a neglected resource for arid lands. *Science* **187** : 803-809.

20

21 **Mitchell PJ, O’Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA.**

22 **2013.** Drought response strategies define the relative contributions of hydraulic dysfunction

23 and carbohydrate depletion during tree mortality. *New Phytologist* **197** : 862-872.

24

- 1 **Mooney HA, Dunn L. 1970.** Photosynthetic systems of Mediterranean-climate shrubs and  
2 trees of California and Chile. *American Naturalist* **104** : 447-453.
- 3
- 4 **Morris H, Plavcová L, Cvecko P et al. 2016.** A global analysis of parenchyma tissue  
5 fractions in secondary xylem of seed plants. *New Phytologist* **209** : 1553-1565.
- 6
- 7 **Nakajima H, Kume A, Ishida M, Ohmiya T, Mizoue N. 2011.** Evaluation of estimates of  
8 crown condition in forest monitoring: Comparison between visual estimation and automated  
9 crown image analysis. *Annals Forest Science* **68** : 1333–1340.
- 10
- 11 **O’Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A. 2014.** Drought survival of  
12 tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate*  
13 *Change* **4** : 1–5.
- 14
- 15 **Orme D, Freckleton R, Thomas G et al. 2013.** CAPER: Comparative Analyses of  
16 Phylogenetics and Evolution in R. R package version 0.5. *Methods in Ecology and*  
17 *Evolution* **3** : 145-151.
- 18
- 19
- 20 **Palacio S, Maestro M, Montserrat-Martí G. 2007.** Relationship between shoot-rooting and  
21 root-sprouting abilities and the carbohydrate and nitrogen reserves of Mediterranean dwarf  
22 shrubs. *Annals of Botany* **100** : 865–874.
- 23

- 1 **Palacio S, Hernández R, Camarero, JJ, Maestro-Martí M. 2012.** Fast replenishment of  
2 initial carbon stores after defoliation by the pine processionary moth and its relationship to  
3 the re-growth ability of trees. *Trees* **26** : 1627–1640.
- 4
- 5 **Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in  
6 R language. *Bioinformatics* **20** : 289–290.
- 7
- 8 **Pérez-Harguindeguy N, Díaz S, Garnier E *et al.* 2013.** New handbook for standardised  
9 measurement of plant functional traits worldwide. *Australian Journal of Botany* **61** : 167-234.
- 10
- 11 **Petri MD, Collins SL, Swann AM, Ford PL, Litvak ME. 2015.** Grassland to shrubland  
12 state transitions enhance carbon sequestration in the northern Chihuahuan Desert. *Global*  
13 *Change Biology* **21** : 1226-1231.
- 14
- 15 **Pivovarovoff AL, Pasquini SC, De Guzman ME, Alstad KP, Stemke JS, Santiago LS.**  
16 **2016.** Multiple strategies for drought survival among woody plant species. *Functional*  
17 *Ecology* **30** : 517–526.
- 18
- 19 **Poorter L, Wright SJ, Paz H *et al.* 2008.** Are functional traits good predictors of  
20 demographic rates? Evidence from five Neotropical forests. *Ecology* **89** : 1908–1920.
- 21
- 22 **Poorter H, Niinemets, Ü, Poorter L, Wright IJ, Villar F. 2009.** Causes and consequences  
23 of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182** : 565–588.
- 24



- 1 **Poyatos R, Aguiadé D, Galiano L, Mencuccini M, Martínez-Vilalta J. 2013.** Drought-  
2 induced defoliation and long periods of near-zero gas exchange play a key role in  
3 accentuating metabolic decline of Scots pine. *New Phytologist* **200** : 388-401.
- 4
- 5 **Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J. 2013.** Dynamics of non-  
6 structural carbohydrates in three Mediterranean woody species following long-term  
7 experimental drought. *Frontiers in Plant Science* **4**, Art No. 400, doi:  
8 10.3389/fpls.2013.00400
- 9
- 10 **Sala A, Hoch G. 2009.** Height-related growth declines in ponderosa pine are not due to  
11 carbon limitation. *Plant Cell Environment* **32** : 22-30.
- 12
- 13 **Sala A, Piper F, Hoch G. 2010.** Physiological mechanisms of drought-induced tree mortality  
14 are far from being resolved. *New Phytologist* **186** : 274-281.
- 15
- 16 **Sala A, Woodruff DR, Meinzer FC. 2012.** Carbon dynamics in trees: feast or famine? *Tree*  
17 *Physiology* **32** : 764-775.
- 18
- 19 **Sala A, Mencuccini M. 2014.** Plump trees win under drought. *Nature Climate Change* **4** :  
20 666–667.
- 21
- 22 **Sapes G, Serra-Diaz, JM, Lloret F. 2017.** Species climatic niche explains drought-induced  
23 die-off in a Mediterranean woody community. *Ecosphere* **8** : e01833.
- 24

- Sarris D, Christodoulakis D, Körner C. 2007.** Recent decline in precipitation and tree growth in the eastern Mediterranean. *Global Change Biology* **13** : 1287-1300.
- Saura-Mas S, Lloret F. 2007.** Leaf and Shoot Water Content and Leaf Dry Matter Content of Mediterranean woody species with different post-fire regenerative strategies. *Annals of Botany* **99** : 545-554.
- Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. 2012.** A Practical Guide to Calculating Cohen  $s^2$ , a Measure of Local Effect Size, from PROC MIXED. *Frontiers in Psychology* **3** : 111.
- Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT. 2014.** How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell Environment* **37** : 153–61.
- Shipley B. 2010.** *From plant traits to vegetation structure*. Cambridge University Press, Cambridge, UK.
- Shipley B, Lechowics MJ, Wright I, Reich PB. 2006.** Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* **87** : 535–541.
- Skelton RP, Westa AG, Todd E, Dawson TE. 2015.** Predicting plant vulnerability to drought in biodiverse regions using functional traits. *Proceedings National Academy of Sciences USA* **115** : 5744–5749.

1  
2 **Terradas J. 1986.** El paisatge vegetal dels Monegros: assaig d'interpretació. *Orsis* **2** : 71-95.

3  
4 **Trumbore S, Czimczik CI, Sierra CA, Muhr J, Xu X. 2015.** Non-structural carbon  
5 dynamics and allocation relate to growth rate and leaf habit in California oaks. *Tree*  
6 *Physiology* **35** : 1206-1222 .

7  
8 **Tyree MT, Engelbrecht BMJ, Vargas G, Kursar TA. 2003.** Desiccation tolerance of five  
9 tropical seedlings in Panama. Relationship to a field assessment of drought performance.  
10 *Plant Physiology* **13** : 1439–1447.

11  
12 **vanWilgen BW, Cowling RM, Burgers CJ. 1996.** Valuation of ecosystem services.  
13 *Bioscience* **46** :184-189.

14  
15 **Vaz M, Pereira JS, Gazarini LC, David TS, David JS, Rodrigues A, Maroco J, Chaves**  
16 **MM. 2010.** Drought-induced photosynthetic inhibition and autumn recovery in two  
17 Mediterranean oak species (*Quercus ilex* and *Quercus suber*). *Tree Physiology* **30** : 946-956.

18  
19 **Venturas MD, Mackinnon ED, Dario HL, Jacobsen AL. 2016.** Chaparral shrub hydraulic  
20 traits, size, and life history types relate to species mortality during California's historic  
21 drought of 2014. PLoS One 11: e0159145. doi:10.1371/journal.pone.0159145.

22  
23 **Vicente-Serrano SM, Beguería S, López-Moreno JI. 2010.** A multiscalar drought index  
24 sensitive to global warming: The standardized precipitation evapotranspiration index. *Journal*  
25 *of Climate* **23**: 1696–1718.

- Vicente-Serrano S, Zouber A, Lasanta T, Pueyo Y. 2012.** Dryness is accelerating degradation of vulnerable shrublands in semiarid Mediterranean environments. *Ecological Monographs* **82** : 407–428.
- Webb CO, Ackerly DD, Kembel SW. 2008.** Phylocom: software for the analysis of phylogenetic community structure and character evolution. *Bioinformatics* **24** : 2098-2100.
- Webb CO, Donoghe MJ. 2004.** Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Resources* **5** : 181-183.
- Westoby M, Reich PB, Wright I. 2013.** Understanding ecological variation across species: area- based vs mass-based expression of leaf traits. *New Phytologist* **199** : 322–323.
- Woodruff DR, Meinzer FC. 2011.** Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. *Plant Cell Environment* **11** : 1920-1930.
- Wright IJ, Reich, PB, Westoby M *et al.* 2004.** The worldwide leaf economics spectrum. *Nature* **12** : 821–827.
- Zhang J, Pei N, Mi X. 2012.** phylotools: Phylogenetic tools for Eco-phylogenetics. <https://rdrr.io/cran/phylotools/man/phylotools-package.html>.
- Zunzunegui M, Barradas MD, Ain-Lhout F, Clavijo A, Novo FG. 2005.** To live or to survive in Doñana dunes: adaptive responses of woody species under a Mediterranean climate. *Plant and Soil* **273** :77-89.

Table 1. Description of studied species. Values of height, SLA, and wood density were obtained as explained in Methods. Values of mean green canopy and plant density were obtained from Sapes et al (2017), except for *P. halepensis*, *L. fruticosum* and *T. tinctoria*. For these three species, mean green canopy was obtained from 50 plants randomly selected from the sampled population, while values of plant density were obtained from five 100m<sup>2</sup> plots located in the study area. The biogeographical range description follows Bolòs et al., 2005 (W: Western, SW: Southwestern).

Species	Family	Biogeographical range	Mean green canopy (%)	Plant density (ind/ha)	Height (cm)	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Wood density (mg mm <sup>-3</sup> )
<i>Pinus halepensis</i>	Pinaceae	Mediterranean	67.2	20.0	690	57.6	0.647
<i>Juniperus phoenicia</i>	Cupressaceae	Mediterranean	40.7	343.6	172	23.3	0.723
<i>Quercus coccifera</i>	Fagaceae	W Mediterranean	71.0	30.4	165	59.7	0.726
<i>Rhamnus lycioides</i>	Rhamnaceae	SW Mediterranean	64.6	172.4	136	91.0	0.763
<i>Genista scorpius</i>	Fabaceae	W Mediterranean	50.2	1443.6	52	134.8	0.765
<i>Rosmarinus officinalis</i>	Lamiaceae	Mediterranean	49.6	4164.0	93	69.4	0.663
<i>Thymus vulgaris</i>	Lamiaceae	W Latemediterranean	68.2	4308.8	21	124.7	0.670
<i>Lithospermum fruticosum</i>	Boraginaceae	W Mediterranean	32.9	116.0	37	129.6	0.666
<i>Thymelaea tinctoria</i>	Thymelaceae	Latemediterranean	76.6	36.0	38	74.5	0.627

Table 2. Results of GLMMs of non-structural carbohydrate (NSC) and soluble sugar (SS) concentrations in the drought (April 2012) and post-rain (February 2013) periods as a function of organ (stem, root), dieback state (undefoliated, with leaf loss), species, and second-level interactions between these factors. NSC and SS values were log-transformed. Cohen's  $f^2_b$  effect size was calculated following Selya (2012).

	Drought					
	NSC ( $R^2 = 0.906$ )			SS ( $R^2 = 0.787$ )		
	F	P	$f^2_b$	F	P	$f^2_b$
Organ	33.56	<b>&lt;0.001</b>	0.2527	1.65	0.201	0.0039
Dieback state	22.55	<b>&lt;0.001</b>	0.0135	3.71	0.056	0.0016
Species	101.18	<b>&lt;0.001</b>	-0.0336	68.39	<b>&lt;0.001</b>	-0.0094
Organ x Dieback	0.28	0.596	-0.0068	0.51	0.477	-0.0023
Organ x Species	12.01	<b>&lt;0.001</b>	0.6070	3 456	<b>0.001</b>	0.1340
Dieback x Species	2.46	<b>0.016</b>	0.0199	3.14	<b>0.003</b>	0.0226

	Post-rain					
	NSC ( $R^2 = 0.842$ )			SS ( $R^2 = 0.803$ )		
	F	P	$f^2_b$	F	P	$f^2_b$
Organ	32.67	<b>&lt;0.001</b>	0.2126	0.20	0.659	-0.0100
Dieback state	2.76	0.099	0.0014	5.20	<b>0.024</b>	0.0051
Species	66.80	<b>&lt;0.001</b>	0.0191	64.19	<b>&lt;0.001</b>	-0.1043
Green canopy	25.82	<b>&lt;0.001</b>	-0.0157	15.40	<b>&lt;0.001</b>	0.0071
Organ x Dieback	3.62	0.059	0.0191	1.75	0.188	0.0012
Organ x Species	9.63	<b>&lt;0.001</b>	0.4074	4.43	<b>&lt;0.001</b>	0.2062
Dieback x Species	0.49	0.65	-0.0017	0.28	0.971	0.0113

Table 3. Results of GLMMs of non-structural carbohydrate (NSC) and soluble sugar (SS) concentration as a function of period (drought, post-rain), organ (stem, root), dieback state (undefoliated, with leaf loss), species, and second-level interactions between these factors. NSC and SS values were log-transformed. Cohen's  $f^2_b$  effect size was calculated following Selya (2012).

	NSC ( $R^2 = 0.863$ )			SS ( $R^2 = 0.780$ )		
	F	P	$f^2_b$	F	P	$f^2_b$
Period	10.27	<b>&lt;0.001</b>	-0.0026	27.82	<b>&lt;0.001</b>	0.0002
Organ	59.60	<b>&lt;0.001</b>	0.1929	0.08	0.781	-0.0026
Dieback state	24.52	<b>&lt;0.001</b>	0.0012	2.82	0.094	0.0006
Species	145.40	<b>&lt;0.001</b>	-0.0008	129.74	<b>&lt;0.001</b>	-0.0503
Period x Organ	1.85	0.174	0.0030	2.41	0.122	0.0011
Period x Dieback	0.43	0.512	0.1974	0.85	0.358	0.0011
Period x Species	1.44	0.180	0.0091	3.60	<b>&lt;0.001</b>	-0.0006
Organ x Dieback	0.76	0.385	0.0019	2.41	0.122	-0.0031
Organ x Species	17.08	<b>&lt;0.001</b>	0.3971	6.30	<b>&lt;0.001</b>	0.1354
Dieback x Species	1.91	0.057	0.0019	2.58	<b>0.010</b>	0.0030

1 Figure 1. (A) Location of the study site. (B) Standardized Precipitation-Evapotranspiration  
 2 Index (SPEI) for years 1951 to 2013, calculated using a time scale of 12 months. Negative  
 3 values (black) indicate water deficit (i.e. drought periods) while positive ones (grey) indicate  
 4 water surplus relative to reference climatic conditions. Each SPEI value is calculated from  
 5 the data of the last 12 months. (C) Difference between historical (1951-2000) and current  
 6 (2009-2013) mean monthly temperature and precipitation. Arrows indicate the two sampling  
 7 dates (drought in April 2012 and post-rain in February 2013). Climatic data corresponds to  
 8 Zaragoza Airport, 90 km west of the Valcuerna study site. Source: Agencia Española de  
 9 Meteorología (AEMET), Ministerio de Agricultura, Alimentación y Medio Ambiente,  
 10 Gobierno de España.

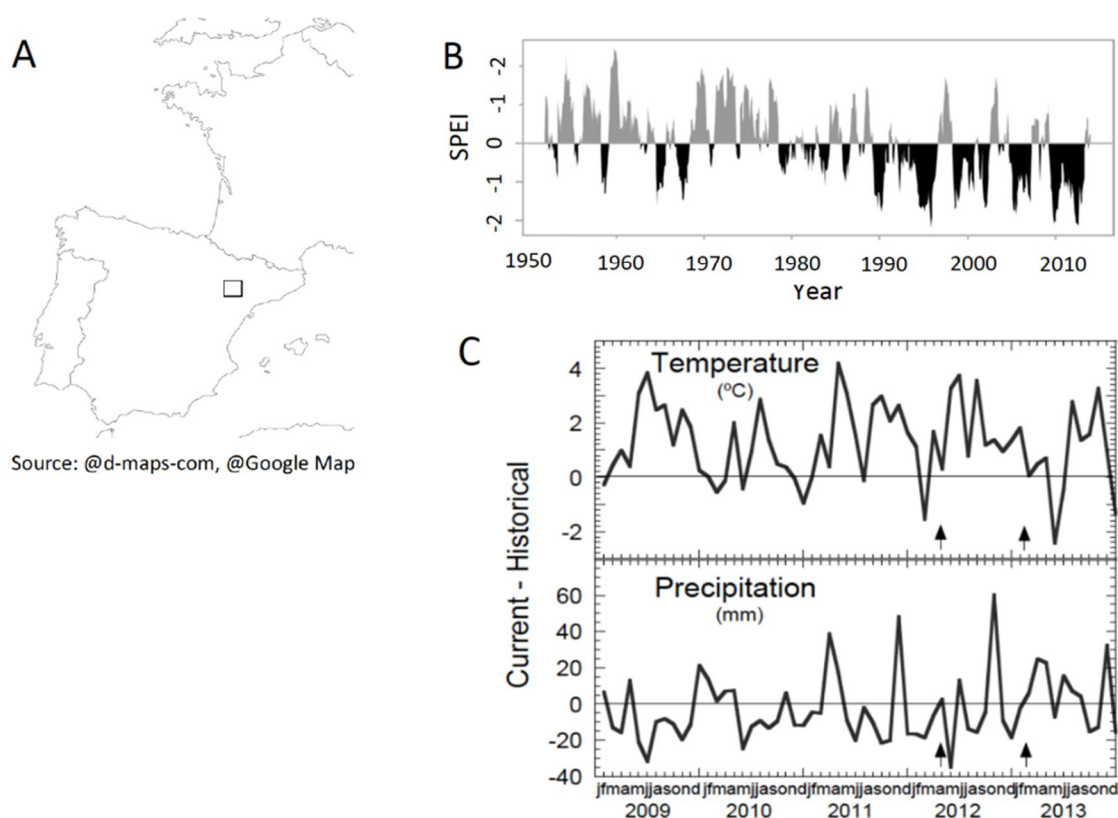




Figure 2. Changes between the drought (April 2012) and the post-rain (February 2013) periods of non-structural carbohydrate (NSC) and soluble sugar (SS) concentrations of the different species in stems and roots of plants with leaf loss and in undefoliated plants. Bold lines correspond to average values of all nine species (see Supplementary information - Figure 1 for detailed information for each species). + or - symbol indicates significant increase or decrease ( $P < 0.001$ ), respectively, between periods, overall considering undefoliated plants and plants with leaf loss; asterisks indicate significant differences ( $P < 0.001$ ) between undefoliated plants and plants with leaf loss, overall considering drought and post-rain periods (ns: no significant differences) (Table 3).

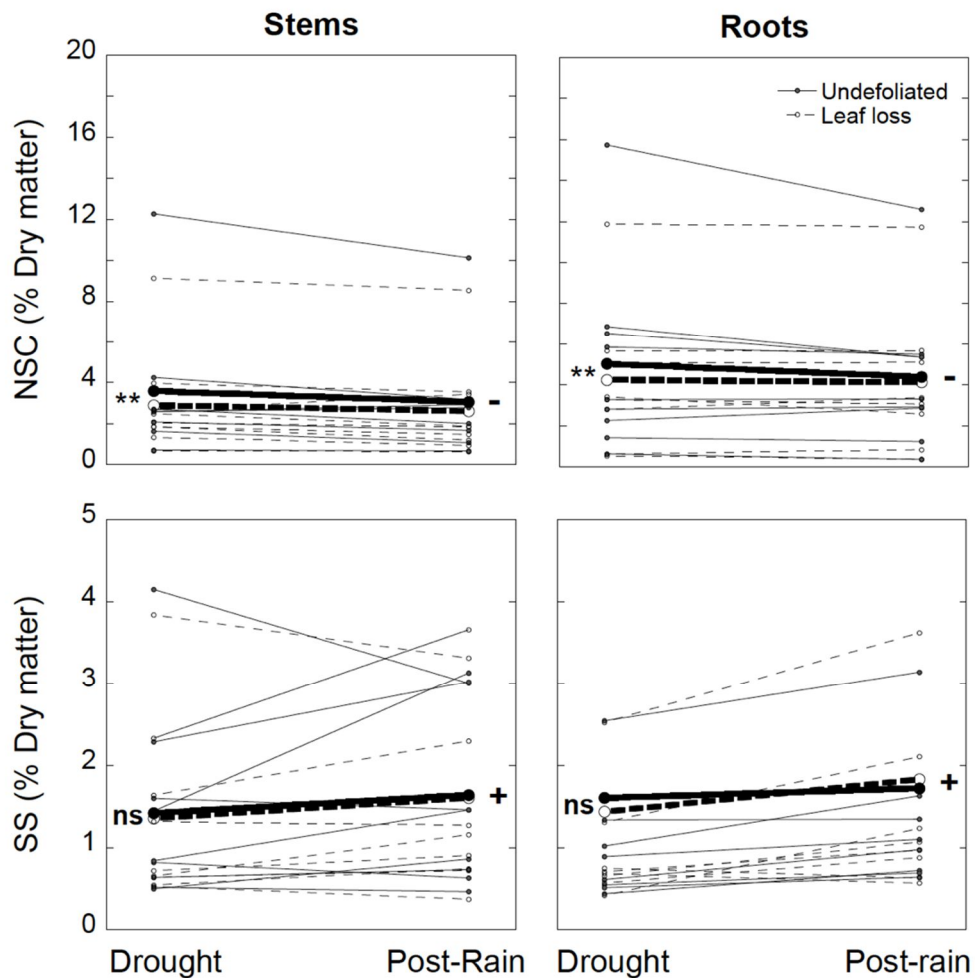


Figure 3. Mean percentage of green canopy during the drought (April 2012) and post-rain (February 2013) periods of plants from the different species. Values were obtained from the plants that were sampled in the post-rain period (N=10 for each dieback state category). Error bars indicate SE. Asterisks indicate significant differences between the drought and the post-rain periods (\*:  $P<0.05$ , \*\*:  $P<0.01$ ). Differences between undefoliated plants and those with leaf loss (dieback) were always significantly different ( $P<0.01$ ).

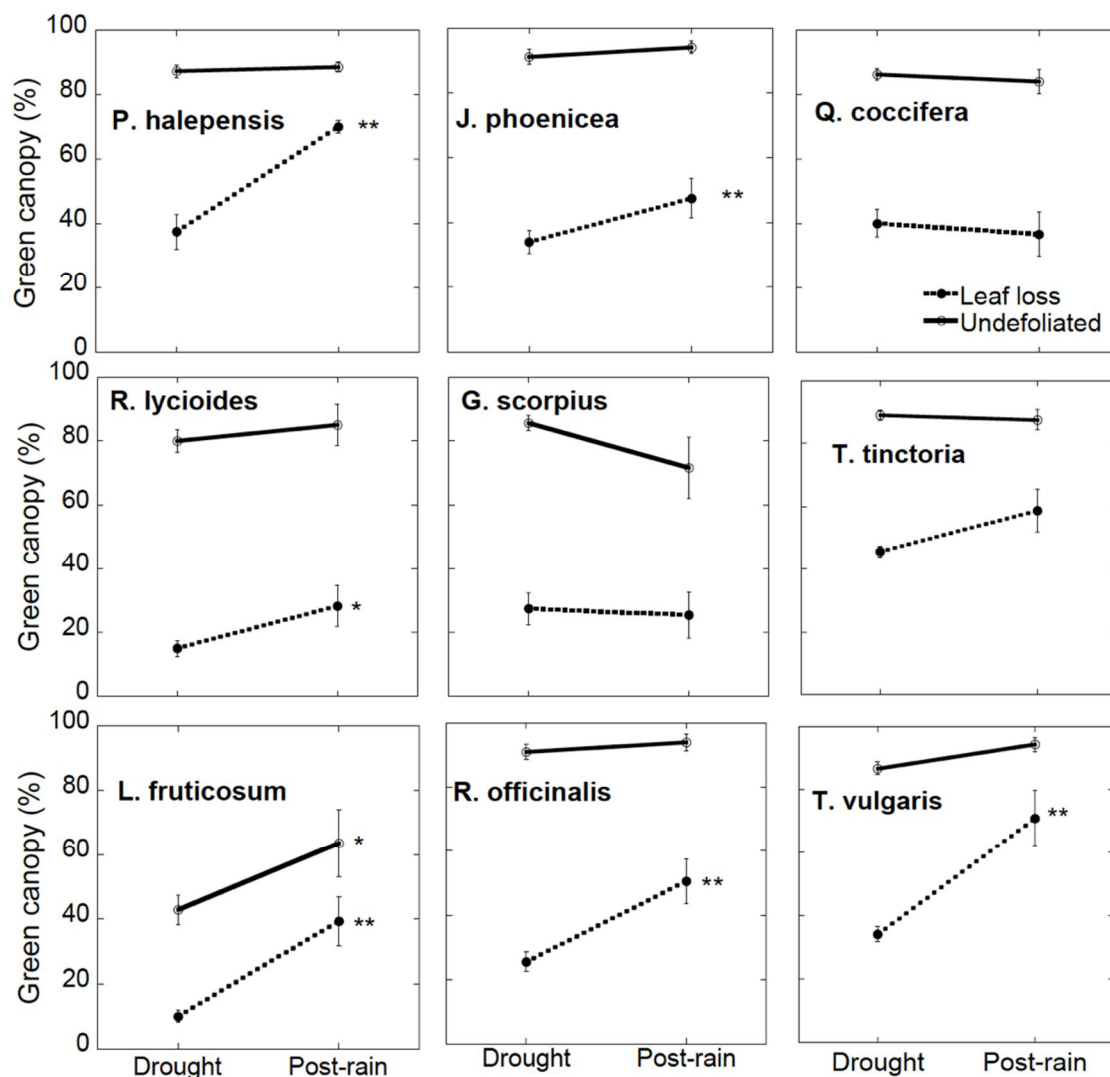


Figure 4. Relationship between change in green canopy (difference in percentage between the post-rain and drought periods) and (A) mean non-structural carbohydrates (NSC) (left panels) and soluble sugars (SS) (right panels) during the drought period, and (B) change in NSC (left panels) and SS (right panels) between periods. Upper panels correspond to stems and lower panels to roots. NSC and SS values correspond to % dry matter content. Open and filled circles correspond to undefoliated and plants with leaf loss, respectively. Only statistical significant relationships are shown (Spearman's non-parametric correlation,  $P < 0.05$ ). Ph: *P. halepensis*, Jp: *J. phoenicea*, Qc: *Q. coccifera*, Rl: *R. lycioides*, Gs: *G. scorpius*, Ti: *T. tinctoria*, Lf: *L. fruticosum*, Ro: *R. officinalis*, Th: *T. vulgaris*. Species abbreviations in italics indicate plants with leaf loss.

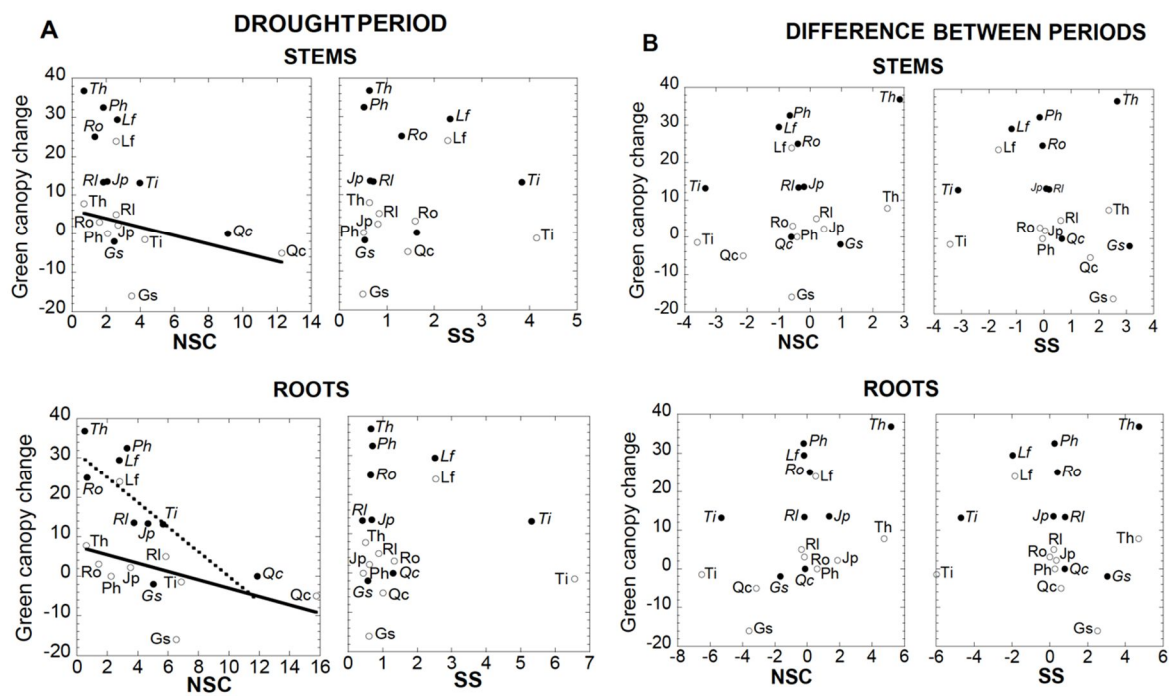
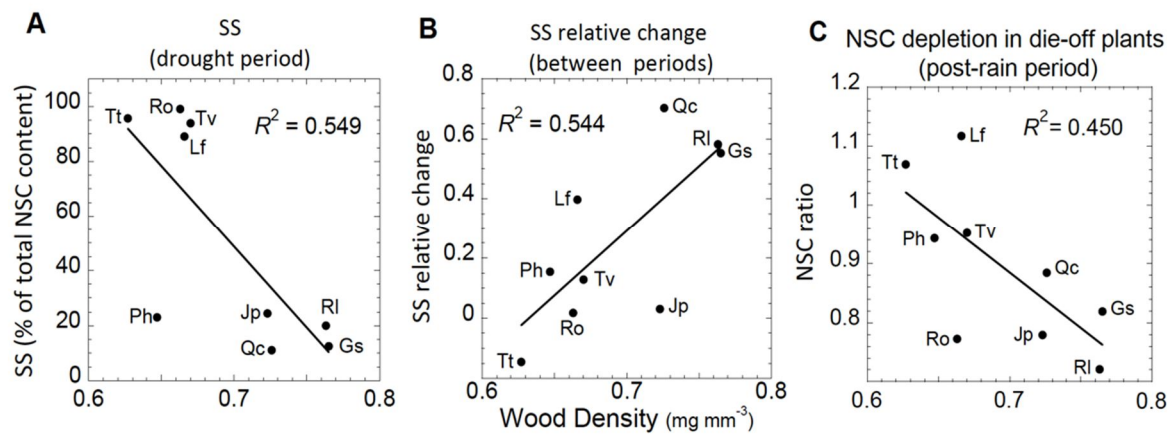


Figure 5. Relationship between wood density (WD) and (A) soluble sugars (SS) during the drought period (as a percentage of total non-structural carbohydrates, NSC); (B) SS relative change between post-rain and drought periods (value in the post-rain period minus value in the drought period / value in the drought period), and (C) NSC depletion in plants with leaf loss, estimated as the ratio of NSC between undefoliated and plants with leaf loss in the post-rain period for each species. NSC and SS values were obtained averaging stem and root values of all undefoliated and plants with leaf loss.  $R^2$  values correspond to linear correlation coefficients. Species abbreviations as in Fig. 4.



**Supplementary Information Table 1.** Mean (SE) non-structural carbohydrates (NSC) and soluble sugars (SS) content (% dry matter) in stems and roots of unaffected and plants with leaf loss (dieback) of the overall set of species during the drought (April) and post-rain (February) periods.

		NSC		SS	
		Drought	Post-rain	Drought	Post-rain
With leaf loss					
	Stems	3.03(0.33)	2.53(0.29)	1.40(0.14)	1.58(0.15)
	Roots	4.32(0.45)	4.03(0.44)	1.48(0.20)	1.81(0.21)
Unaffected					
	Stems	3.64(0.45)	3.12(0.44)	1.47(0.14)	1.66(0.13)
	Roots	5.06(0.59)	4.70(0.44)	1.59(0.23)	1.73(0.18)

**Supplementary Information Figure 1.** (A) Non-structural carbohydrate (NSC) content and (B) soluble sugar (SS) of the different species in stems and roots of undefoliated plants and those with leaf loss (dieback) during the drought (April 2012) and post-rain (February 2013) periods. Asterisks indicate significant differences between undefoliated and plants with leaf loss of the same species in a given period (\*:  $P<0.05$ , \*\*:  $P<0.01$ ). Bold lines indicate significant differences ( $P<0.05$ ) between the two periods. NSC and SS values correspond to % dry matter content.

