Trees (2015) 29:1791–1804
DOI 10.1007/s00468-015-1261-9

ORIGINAL ARTICLE

Pine mortality in southeast Spain after an extreme dry and warm year: interactions among drought stress, carbohydrates and bark beetle attack

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Received: 9 February 2015 / Revised: 14 July 2015 / Accepted: 17 July 2015 / Published online: 29 July 2015

Abstract

Key message **Pine mortality was related to water stress, which caused xylem cavitation.Hydraulic failure and carbon starvation are likely interrelated, and bark beetles attacks did not seem to be directly involved.**

Abstract Forests are extremely important for society given the many services they provide. Climate models reflect increases in temperature and less annual rainfall, which will generate hotter drier environments. Under these conditions, it is predicted that forest ecosystems will be severely affected, and recent studies have accumulated evidence for drought-induced tree mortality. Consequently, many studies have attempted to explain mechanisms of survival and mortality in forest species. However, the physiological mechanisms that underlie drought mortality are not completely understood. The aim of the present study was to analyse the effect of an extremely dry year on the cause of mortality of pines and on forest decline in pine forest populations in southeast Spain. Specifically, we studied the effect of drought stress that caused pine mortality, dynamics of carbohydrates reserves and bark beetle attack. The results suggest that pine mortality can be attributed to an intense drought stress level that caused xylem cavitation. The results also indicate that hydraulic failure and carbon starvation are likely interrelated, which makes separating both mechanisms very difficult. Finally, the recorded bark beetles attack did not seem to be directly involved in mortality, at least not in the forests with less intense drought conditions.

Keywords Pine mortality · Xylem cavitation · Carbohydrates · Bark beetle infestation · Drought · Temperature anomalies

Introduction

Forests are extremely important to society for the many services they provide. Everyone worldwide either depends directly on forests for their livelihood or indirectly benefits from them. As 21st century climate models reflect, temperatures may rise by 3–4 °C and annual rainfall may diminish (IPCC 2013). Future climate warming is expected to increase atmospheric water demand, reduce soil water availability and generate hotter drier environments in the east Mediterranean region (Bates et al. 2008). However, changes will not be restricted to increases in mean temperature, but will also involve greater drought frequency and intensity (Sterl et al. 2008). Under these conditions, forest ecosystems will be severely affected by relatively rapid climate changes. As a result, some species will be favoured. However, others will be unable to adapt or migrate fast enough and may, therefore, become extinct (Peñuelas et al. 2007).

Communicated by H. Rennenberg.

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Electronic supplementary material The online version of this article (doi:10.1007/s00468-015-1261-9) contains supplementary material, which is available to authorised users.

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These features lead to increased water stress in plants, and consequently to a high risk of plant mortality events during intense dry periods in summer. In fact, several studies have provided evidence for drought-induced tree mortality (Allen et al. 2010; Hartmann et al. 2015 and references therein). This increased mortality trend, associated with periods of drought or extreme heat, has already been observed for all biomes and plant functional types (Allen et al. 2010), and also for declining forest productivity (Dorman et al. 2013), stunted tree growth (Leuzinger et al. 2005) and greater sensitivity to attacks by biotic agents (McDowell et al. 2011).

Numerous theories have been put forward to explain the survival and mortality mechanisms observed in recent years. However, the physiological mechanisms that underlie drought mortality are not completely understood, and can be related to severe reductions in carbon reserves in some cases (non-structural carbohydrates), and to hydraulic failure in others (McDowell et al. 2011). Other works have suggested that these two processes might be complementary (McDowell et al. 2011; Vilagrosa et al. 2012). Under dry conditions, negative water potentials tend to cause xylem tension, and severe water stress can produce cavitation and the embolism of vessels and tracheids. These embolisms reduce water transport capacity in xylem and cause a lower leaf water potential, which once again produces more embolisms (Tyree and Zimmermann 2002). Under severe drought conditions, this cycle can become unstable (Meinzer et al. 2009) and can break the hydraulic continuum between soil and plants, and can consequently lead to plant mortality (Vilagrosa et al. 2010). The xylem embolism can be an important ecological factor, not only because it directly reduces the plant potential for gas exchange (Sperry et al. 2002), but also because it can limit the minimum water potential that plants tolerate.

However, another theory has suggested that tree mortality may also be caused by continuous plant carbon reserve depletion due to stomatal regulation (McDowell et al. 2008). In general, maintaining a positive carbon (C) balance during drought is often considered a major challenge for trees (Mitchell et al. 2013). The classical carbon starvation hypothesis predicts that stomatal closure, which prevents hydraulic failure, causes photosynthetic carbon uptake to diminish, and plants starve as a result of continued metabolic demand for carbohydrates. This process may be exacerbated by either photoinhibition processes or more respiratory demands, associated with high temperatures during drought (Klein et al. 2011). Apart from the above-mentioned causes, plant mortality can be exacerbated by biotic attacks. Several studies have reported a positive relationship between droughts and insect attacks or other pathogens on trees, and both factors have been related to mortality events (Gaylord et al. 2013).

One of the most frequently found forests in the Mediterranean region is Aleppo pine (*Pinus halepensis*) forest, which cover over 25,000 km^2 in the Mediterranean basin (Quézel 2000). This is partly because this species has traditionally been the most widely planted species in reforestation programmes in southeast Spain (Pausas et al. 2004). As a result, it covers extensive areas in the driest part of Mediterranean ecosystems, and is considered potential vegetation in almost 50 % of national forest territory (Vélez 1986; Pausas et al. 2004). In Spain, 3.8 million hectares were reforested during the 1945–1986 period, and 90 % of the reforested area was planted with pines (ICONA 1989). Similar observations have been made in semiarid regions of Turkey, Algeria and Morocco (Barbéro et al. 1998; Pausas et al. 2004). From a functional point of view, *Pinus halepensis* is considered not only a drought-avoider isohydric species with efficient stomatal control over water use (Baquedano et al. 2008), but also a species that displays moderate resistance to the xylem embolism compared to other *Pinus sp* (Choat et al. 2012).

The aim of the present study was to analyse the effect of an extremely dry year on the mortality of pines and on forest decline in several pine forest populations in southeast Spain. We attempted to analyse (i) if drought conditions determined mortality by lowering the water potential and if they produced cavitation of xylem conduits; (ii) why some trees survived, but others showed marked signs of decline and leaf desiccation; (iii) the role of carbohydrates between living and declining pines; (iv) the chances of functional recovery after autumn precipitations. To analyse these questions, we determined the level of water potential, xylem cavitation, leaf photochemical efficiency and non-structural carbohydrates in four pine forests. We then related these variables with impact of drought in terms of the pine mortality, and the 2014 records of rainfall and temperature in each forest. These measures were taken in four Aleppo pine (*Pinus halepensis*) forests in the province of Alicante (southeast Spain), one of the Iberian Peninsula sites that is more intensely affected by degradation and desertification risks. The underlying hypothesis of this study was that a severe drought during the current year would favour pine mortality promoted mainly by bark beetle attack but, alternatively, xylem cavitation and carbohydrate starvation could result in mortality prior to a subordinate beetle attack.

Materials and methods

Study sites and plant material

The study was carried out in four *Pinus halepensis* forests located in southeast Spain (province of Alicante) on a

Table 1 Main characteristics of the pine forests studied in the present work

Forests	Catí	San Juan	Albatera	Orihuela
Plot location	38°31′N, 0°41′W	38°24′N, 0°27′W	38°13′N, 0°53′W	38°5′N, 0°56′W
Surface (m^2)	3427	3871	1614	3417
Slope aspect	S	SE	S	SE
Historical averaged temperature (°C)[a]	14.2	17.9	18.2	18.1
Historical averaged precipitation (L m^{-2})[a]	323	284	279	297
Tree density (pines per ha)	100	500	700	400
Basal diameter (m)	0.155 ± 0.014	0.117 ± 0.004	0.123 ± 0.009	0.171 ± 0.014
Pine height (m)	4.09 ± 0.27	2.13 ± 0.12	3.13 ± 0.16	3.86 ± 0.16
CEAM weather stations[b]	Villena	Villena/Albatera	Albatera	Orihuela

AEMET (National Weather Service) weather stations were taken as references given their large dataset collections

[a] Data from Spanish government weather stations (AEMET) with large datasets (as reference historical data)

[b] CEAM weather stations close to the forest sites with datasets covering 2004–2013

north–south gradient, which also corresponded to a precipitation and temperature gradient. From north to south, the forest plots were Catí, San Juan, Albatera and Orihuela (Table 1). The climatic characteristics correspond to a semiarid climate with an annual average precipitation of 200–350 L m^{-2} (Pérez Cueva 1994). Soils were developed mainly from marly bedrock, with a high content of carbonates, a slightly basic pH, a low organic matter content and a generally low fertility level.

The study was carried out in late summer and at the beginning of autumn in 2014 after an extremely intense summer and a long dry period. The selected forests have resulted from old pine reforestations, whose current age range is 40–60 years old. In each forest, living pines with an apparently healthy condition (green leaves) and declining pines with evident decay symptoms (bent leaves, most were completely withered or brown) were selected. No tree with declining symptoms was found in the Catí forest. To establish pine characteristics, height, basal diameter and tree density were measured for 10–15 pines in each study area (Table 1).

In all four forests, pine mortality was determined by field visits and by counting the number of the trees that had recently died to then compare them to the remaining living trees. A tree was considered dead when all its leaves were brown. The data used for this study were collected at the end of November.

Meteorological data

Annual precipitation and monthly temperature for each forest are shown in Table 1. The average values were assigned from the nearest thermo-pluviometric stations (AEMET weather stations net) and values are the averages for the 1961–1990 period (Pérez Cuevas 1994).

The 2014 data correspond to the hydrological year (August 2013 to September 2014) and came from the CEAM's network of automatic stations. These stations were selected for their proximity to each forest. CEAM weather stations are equipped with sensors, which are used to measure the main atmospheric parameters. For temperature, Vaisala sensors (50Y and HMP45A Humitter, Vaisala, Helsinki, Finland) were used. Rain Gauges (models MT 7856 and 52203, Davis Instruments, CA, USA) were employed for precipitation. Meteorological stations took recordings over short time intervals (10 min), and data were stored in a data logger (Campbell Scientific CR10X model, CR510, CR800 and CR1000, and brand Coastal Environmental Ltd, Zeno 3200 model). Data transmission was performed via modem (communication system GSM and GPRS Siemens TC65), and data were checked on daily and monthly bases.

Water potentials, leaf water content and chlorophyll fluorescence measurements in living and declining trees

Water potentials (WP) were measured early in the morning, shortly after predawn, in mid-September by a Scholander pressure chamber (Model 1000, Pressure Chamber, Instrument, PMS Instrument Company, USA). Samples were placed inside a bag under dark conditions to allow the equilibration of the water potential in twigs. Five pines were selected per site, and one small twig per tree was used to measure the water potential. Small twigs that were representative of the tree were selected. The same protocol was applied for the dead trees at the Albatera, San Juan and Orihuela sites, but all the samples were lower than −7.5 MPa, which is the limit of our pressure chamber. The WP in declining trees were estimated by regression between leaf water content and WP (see Suppl. Mat. 1). Leaf water content (LWC) was calculated as the leaf fresh weight-leaf dry weight/leaf dry weight ratio. A sample of 10–15 leaves per tree was weighed (leaf fresh weight) in the lab on a precision balance (0.1 mg). Later samples were placed inside an oven at 65 °C for 24–48 h until constant weight and then sample weight was determined again (leaf dry weight). LWC was estimated on the same branches as WP and used to estimate the WP in declining trees (see Suppl. Mat. 1).

Maximum photochemical PSII efficiency (Fv/Fm) was measured by a portable pulse-modulated fluorometer (PAM-2100, Walz, Effeltrich, Germany) equipped with a leaf clip holder. Fv/Fm was measured using the same five trees used for measuring WP. One measure was taken per tree on a pack of 10–15 needles. Leaves were pre-darkened for 30 min before measurements started.

Hydraulic conductance and xylem cavitation

Shoot hydraulic conductance and xylem cavitation were determined in the same living and declining trees as in previous measurements, and in five pines per site and tree condition. One-metre-long branches were cut with pole pruners early in the morning (8–9 am, solar time) from mature trees. Branches were enclosed in black airtight plastic bags to reduce water loss through transpiration, and were immediately taken to the laboratory, where stem segments (2–3 cm long approx.) of twigs were cut under water before measuring their hydraulic conductance. Segments were shaved at both ends with a razor blade to be inserted into a tubing manifold which was immersed in water. This tubing manifold was connected to the measuring circuit of the equipment "Xylem embolism–meter" (Xyĺem, Instrutec, Bronkhorst, France). Branch segments were perfused with distilled and degassed water. Specific hydraulic conductance: Ks (kg m^{-1} Mpa-1 s^{-1}) was calculated as the ratio between Kh and the mean cross-sectional area of the segment without bark (Vilagrosa et al. 2003). As *Pinus sp* have tracheids with torus margo, removing the air inside tracheids and restoration of maximum hydraulic conductance ($K_{\max}$) can be very problematic. For this reason, we used the initial Ks values as a parameter to estimate declining hydraulic conductance (i.e. xylem cavitation) compared with other individuals under low levels of stress.

Non-structural carbohydrates (NSC)

Non-structural carbohydrates (NSC) were determined as soluble sugars (SS; i.e. sucrose, glucose and fructose) and starch content (Starch) in sapwood samples at the end of summer and at the same time as the other measurements. Samples were taken in five individuals per forest, which were chosen from the same individuals measured for WP and for the other measures. NSC on living pines were analysed in all four forests, and were only analysed on declining trees in two forests (Albatera and San Juan). A wood sample about 10-cm long without bark from a basal part of a well-developed branch in the medium-upper part of the canopy was taken. Taking samples from leaves was avoided due to the withering condition, and also from the trunk to avoid damage which could condition its future persistence and survival. This part of the tree has been used in several former studies and has provided reliable information about NSC dynamics (Sala and Hoch 2009; Anderegg and Anderegg 2013; Rosas et al. 2013). Samples were frozen in the field with liquid nitrogen in a Dewar container. Then, they were transferred to a freezer and stored at −25° C for further analyses in a specialised laboratory (Agrolab Analítica SL). These analyses allowed us to determine the quantification of stored reserves. In the analytical laboratory, NSC samples were oven-dried at 75 °C and ground in an electrical mill to pass a 1-mm screen. Starch was determined by the enzymatic method (amyloglucosidase and Glucose-oxidase–peroxidase) and was then measured colorimetrically in a visible spectrophotometer at the 500 nm UV wavelength. Soluble sugars (total and reductors) were extracted by reduced ferrocyanide, and their concentration was determined colorimetrically in a visible spectrophotometer at the 540 nm UV wavelength.

Bark beetle attack

Bark beetles of the genera *Tomicus sp* and *Orthotomicus sp* were detected in the forests studied herein. The degree of infestation was determined by a visual analysis of the dead pines at the end of November, during the last field monitoring. A tree was considered affected by bark beetles when the number of holes in its bark exceeded 100, which reflects a high degree of attack. The bark on trees was stripped to confirm the high level of infestation by bark beetles. In each plot, about 20 trees (dead and living) were examined for beetle attack. No evident signs of beetle attack were observed in living trees, which is why they were removed from this analysis.

Drought-recovery analysis

After the autumn rains (mid-November to December), all the declining pines died and drought recovery was analysed in the remaining living pines. In order to analyse pines' capacity for recovery (Δ) after the autumn rains, tWP, Fv/Fm and Ks were analysed by making a comparison between the post-summer and post-autumn rains in November to mid-December. Δ was computed as 1-(minimum value/maximum value) for each sample, and was expressed as a percentage. ΔWP, in absolute values, was computed as the minimum values that corresponded to the WP in November, and the maximum ones that corresponded to post-summer. For ΔFv/Fm and ΔKs, the maximum values were those computed in November, and the minimum ones were the post-summer values. The protocols were the same as those used after the summer determinations.

Statistical analysis

WP, Ks, Fv/Fm, NSC, starch and SS were compared by a two-way ANOVA by taking pine forests (Orihuela, Albatera, San Juan and Catí) and tree condition (living and declining) as the main factors. Living and declining pines for the starch and SS analyses were compared in only two forests (Albatera and San Juan) due to the limitations to analyse carbohydrates. In order to obtain more detailed differences among forests, a one-way ANOVA with pine forests as the factor was applied to study the different variables in detail in some specific analysis as for the capacity for recovery (Δ) and the averaged values after autumn rains. A post hoc Tukey-*B* test at $P < 0.05$ was applied to establish differences among pine forests.

In all the analyses, a Shapiro–Wilk test and a Bartlett test were run to measure the normality and homogeneity of the residuals, respectively. Data were transformed when necessary in order to meet the assumptions of the ANOVA.

A regression analysis of the living pines was done to study the relationships between WP as a proxy of the drought level intensity and the other variables (Fv/Fm, Ks, starch or SS).

Results

Climatic anomalies related to temperature and precipitation

Annual precipitation during the hydrologic 2014 year showed that the total amount of rainfall sharply dropped; and values were 24–40 % of the normal values for these stations (Table 1; Fig. 1). Rainfall was especially low for the Orihuela forest with about 60 mm of total rainfall for that year, compared to the usual common precipitation regime of about 269 mm during a hydrologic year from September to August (Fig. 1a).

The temperature trend showed a hotter 2014 year with increments of about 1–3 °C in spring and autumn, and an increment of 3.5 °C in April. These values imply that those months showed higher temperatures than historical averages (Fig. 1b). Throughout the year, only summer months came close to historical averages.

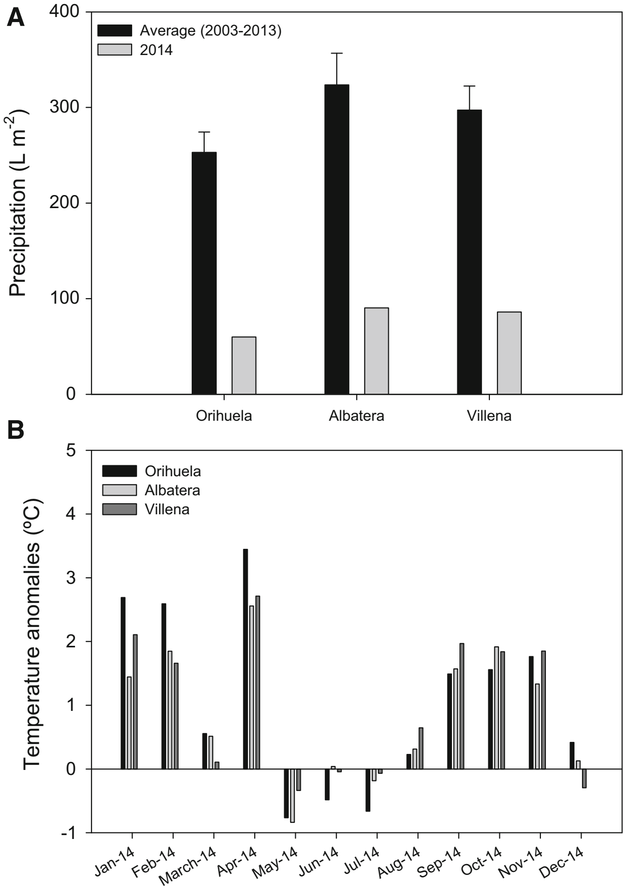

Fig. 1 **a** Annual precipitation during the 2014 hydrologic year (September 2013–August 2014) compared to historical averages (CEAM weather stations, 2004–2013) in the province of Alicante (Spain) from the most humid range (Villena) to the driest site (Orihuela). **b** Temperature anomalies (difference between the monthly average and the historical average) for the same weather stations on a monthly basis. Positive values correspond to hotter months and negative values to colder months compared with the historical average per month (2004–2013) (Source: CEAM)

Pine mortality

Differences for mortality were found among the studied pine forests at the end of November (Fig. 2). Mortality came close to 100 % in Orihuela, but no mortality was observed in Catí. The other two forests, Albatera and San Juan, showed intermediate rates with 18 and 4 % of pine mortality, respectively.

Drought stress levels after summer and comparisons between living and declining pines

The WP of declining trees were significantly lower than that of living trees at the end of summer (Fig. 3; Table 2), with values below −7.7 MPa for declining pines. Significant differences among pine forests were also found, which

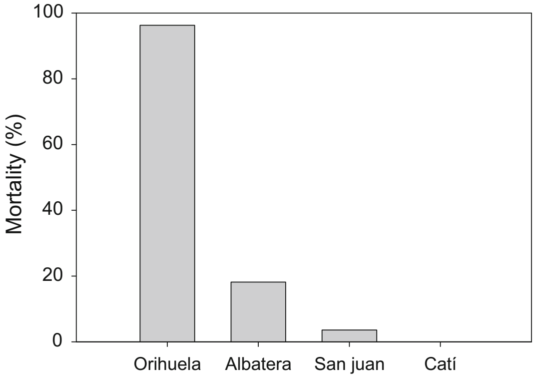

Fig. 2 Mortality rates recorded after summer 2014 for the different Aleppo pine (*Pinus halepensis*) forests. Data correspond to the end of November

reflects a gradient of drought stress. For the living pines, the WP values at the end of summer ranged from −6.0 ± 0.4 to −3.2 ± 0.1 MPa, with significant differences found among populations (Fig. 3; Table 2). The Orihuela forest obtained the lowest WP values, which reflects high levels of drought stress. Forests Catí and San Juan presented the highest values, with intermediate ones for the Albatera forest. Significant differences were observed for declining pines among forests Orihuela, Albatera and San Juan, but the range of values came close to −8 MPa in all four forests (Fig. 3; Table 2).

Differences for Fv/Fm were also observed among forests and between living and declining pines (Fig. 3; Table 2). For living pines, Catí and San Juan obtained the same values, which were significantly higher than those of Albatera and Orihuela (Fig. 3; Table 2). The Fv/Fm values in declining trees were under 0.080, and some minor differences were found among the forests (Fig. 3; Table 2).

For WP and Fv/Fm, an interaction was found between pine forests and tree condition (Table 2). This interaction showed that the response of both variables in each forest did not parallel with the response between living and declining pines (Fig. 3; Table 2). In fact, all declining pines obtained a WP value lower than −7.5 MPa, while the same variables for living pines reflected a water stress gradient among pine forests. The same can be stated of Fv/Fm, where the values for declining pines were always below 0.1 (relative units). This interaction also reflected similar high drought stress levels in declining pines compared to living ones.

Water transport capacity in shoots (Ks) was affected by the drought levels found in each pine forest and tree condition (Fig. 3; Table 2). The highest Ks values fo living pines went to Catí, with 9.8 ± 0.4 kg s^{-1} m^{-2} MPa^{-1} on average, and Orihuela obtained the lowest values, 1.49 ± 0.37 kg s^{-1} m^{-2} MPa^{-1} (Fig. 3, Table 2). San Juan and Albatera presented intermediate values, and no significant differences with Orihuela were observed (Fig. 3; Table 2). These values indicated a major reduction in Ks compared to the maximum values obtained for Catí

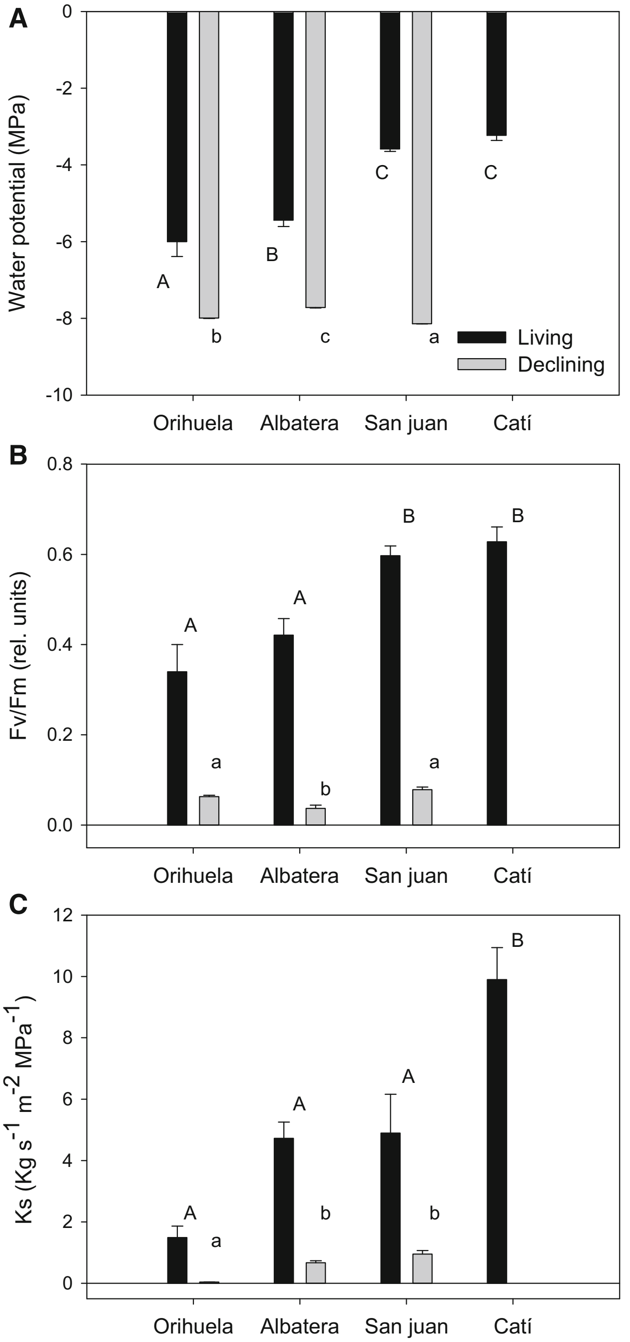

Fig. 3 Comparisons made between living and declining pines in the different pine forests at the end of summer. **a** Water potential (WP), **b** Maximum photochemical efficiency of PSII (Fv/Fm), **c** Hydraulic conductance (Ks) for twigs. Bars are the mean ± SE of $n = 4$–7 individuals for each pine condition and forest. *Different letters* mean significant differences among pine forests at $P < 0.05$ (post hoc Tukey-*B* test). Statistical results correspond to the one-way ANOVA, which found statistical differences among pine forests within each pine condition factor

Table 2 Two-way ANOVA results of the main effects and interactions for the comparisons made among tree conditions (living, declining pines) and forest populations (Orihuela, Albatera, San Juan and Catí)

Source	WP			Fv/Fm			Ks		
	MS	dof	*F*	MS	dof	*F*	MS	dof	*F*
Tree condition (TS)	64.4	1,31	616.1^{***}	1.2	1,31	267.4^{***}	63.8	1,27	20.0^{***}
Pine forests (PF)	6.9	3,31	65.8^{***}	0.08	1,31	18.9^{***}	65.7	3,27	20.6^{***}
TSx PF	5.5	2,31	52.4^{***}	0.38		8.8^{**}	4.5	2,27	1.4^{ns}
Post hoc Tukey-B for PF	Orih < Alb < SJ < Catí			Orih = Alb < SJ < Catí			Orih = SJ < SJ = Alb < Catí		

Differences among forests were established by a post hoc Tukey-*B* test at $P < 0.05$. $N = 4$–7 samples for each tree status and pine forest

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Forests are Orih: Orihuela, Alb: Albatera; SJ: San Juan

with the lowest stress level. The Ks values in declining trees were around 0.607 kg s^{-1} m^{-2} MPa^{-1}, which indicates a sharp drop in hydraulic conductance. Consequently, Ks was considerably lower in declining vs. living trees (Fig. 3; Table 2) for each forest. No interaction was found for this variable, which indicates some proportionally decreasing values from the less stressful conditions (i.e. San Juan forests) to the driest forest (i.e. Orihuela) in both living and declining pines.

Autumn recovery in living pines

In November, after the autumn rains, all the pine forests showed some degree of recovery compared to the previous drought conditions and analysed variables (Tables 3, 4I). Higher WP values were found when compared to the post-summer values (Table 4I). Recovery (i.e. a comparison of the post-summer values to the after-rain values) showed that they were different (Table 4I), but the interaction term was significant. In fact, the Δ rates were not in accordance with the WP values recorded in each forest (Table 4I, II). For ΔWP, significant differences were obtained among all the pine forests, but Albatera and Catí obtained high ΔWP rates (Table 3I). Contrarily, the WP after the autumn rains displayed a different pattern, and the final values were observed in November, where the Orihuela forest obtained the lowest WP values, and Catí had the highest ones. Albatera and San Juan showed intermediate values (Table 3II).

Similarly, Fv/Fm rates significantly recovered in all the forests when compared with the post-summer values (Table 4I). In this case, the ΔFv/Fm for Orihuela and Albatera showed a higher degree of recovery than for San Juan and Catí (Tables 3I, 4II). As in WP, the interaction term showed a different recovery response among the pine forests, where no statistical differences were found (Table 4II) in the Fv/Fm values in November. This indicates that all the trees obtained suitable Fv/Fv values (around 0.76–0.79; Table 3II).

The Ks values after summer and the autumn rains differed significantly (Table 4I), which indicates some degree of xylem conductance recovery. However, the ΔKs analysis found no differences among the pine forests, which suggests that the recovery of Ks was similar in all the forests, and independently of the degree of the post-summer values (Table 3I). ΔKs was similar for Orihuela, Albatera and San Juan, and its values ranged between 25.7 and 39 %. It is noteworthy that Catí showed no recovery (−2.2 ± 11.9 %). The high error terms found in all the forests indicate a high level of variability within each forest which, in turn, suggests that some trees recovered hydraulic conductance, while others continued to show similar values. The results found in November reveal that only a small fraction of hydraulic conductance had been recovered with the autumn rains, especially if we take into account the lower values recorded for the drier forests (Table 3II). In fact, the Catí forest gave similar Ks values to the post-summer and November measures, while San Juan and Albatera displayed significant recovery as the initial Ks values almost doubled after the autumn rains (i.e. from 4.7 to 8.7 kg s^{-1} m^{-2} MPa^{-1}; Tables 3II, 4II). Orihuela was the tree population that exhibited the most marked reduction in xylem conductance after summer (1.49 ± 0.37 kg s^{-1} m^{-2} MPa^{-1}), which recovered, but to a less extent, with values of 2.87 ± 0.33 kg s^{-1} m^{-2} MPa^{-1}. The statistical analysis showed no differences among Albatera, San Juan and Catí for the measures taken after the autumn rains (Table 3II).

Starch and soluble sugars dynamics in living and declining pines

NSC values ranged from the lowest values (0.56 ± 0.07 %) of dry matter measured in Albatera, to the highest value (0.92 ± 0.08 %) taken in San Juan. Statistical results for living pines showed significant differences among forests (Fig. 4; Table 5), but all the values came close to 1 % on a dry matter content basis. Differences in

Table 3 Results of autumn recovery for WP, Fv/Fm and Ks in living pines

	Orihuela	Albatera	San Juan	Catí
I. Δ recovery (%)				
ΔWP	46.7 ± 4.3b	55.8 ± 0.9a	39.9 ± 1.2b	60.8 ± 1.6a
ΔFv/Fm	55.2 ± 8.0a	45.5 ± 5.6a	20.5 ± 3.4b	20.2 ± 4.1b
ΔKs	39.2 ± 24.7	25.7 ± 14.5	39.2 ± 29.9	−2.2 ± 11.9
II. After autumn rains				
WP (MPa)	−3.3 ± 0.2a	−2.4 ± 0.1b	−2.2 ± 0.02b	−1.3 ± 0.1c
Fv/Fm (Rel. units)	0.76 ± 0.003	0.79 ± 0.002	0.76 ± 0.03	0.79 ± 0.002
Ks (kg s^{-1} m^{-2} MPa^{-1})	2.87 ± 0.33a	8.03 ± 1.36b	8.74 ± 1.34b	9.81 ± 0.35b

I Capacity of recovery (Δ) expressed as 1-(minimum values/maximum values) from post-summer values and after autumn rains (see "Materials and methods" section). *II* Values measured in November after the autumn rains. *Different letters* mean significant differences among pine forests at $P < 0.05$ (post hoc Tukey-*B* test). See Table 4 for statistical comparisons. Mean ± SE of $n = 4$–7 samples for each tree condition and pine forest

Table 4 Statistical autumn recovery results in living pines

Source	WP			Fv/Fm			Ks		
	MS	dof	*F*	MS	dof	F	MS	dof	*F*
I. Two-way ANOVA									
Recovery (R)	58.5	1,38	490.2^{***}	0.83	1,37	196.5^{***}	46.1	1,35	9.5^{**}
Pine forests (PF)	12.7	3,38	106.2^{***}	0.05	3,37	12.8^{***}	107.3	3,35	22.1^{***}
R x PF	1.5	3,38	12.4^{***}	0.05	3,37	12.0^{***}	9.4	3,35	1.9^{ns}
Post hoc Tukey-B for PF	Orih < Alb < SJ < Catí			Orih = Alb < SJ = Catí			Orih < Alb = SJ < Catí		
II. One-way ANOVA (for forests factor)									
(i) Recovery capacity (Δ)	0.05	3,18	24.9^{***}	0.15	3,17	11.8^{***}	0.22	3,16	1.2^{ns}
(ii) After autumn rains	4.1	3,20	63.1^{***}	0.002	3,19	1.3^{ns}	50.8	3,18	10.9^{***}

I Two-way ANOVA results (*F* and dof) of the main effects and interactions for the comparisons made among forest populations (Orihuela, Albatera, San Juan and Catí) and recovery factor (comparison of the post-summer values with the values measured after the autumn rains). *II* One-way ANOVA for the individual effects of recovery capacity (Δ) and pine forest. Differences among forests were established by a post hoc Tukey-*B* test at $P < 0.05$

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Forests are *Orih* Orihuela, *Alb* Albatera; *SJ* San Juan

starch and SS were also noted among forests (Table 5I), but always within a short variation range (Fig. 4). No clear tendency was observed among pines, and a diminishing relationship was observed only when SS was expressed in terms of WP (see below).

The impact of drought on tree condition (i.e. comparison between living and declining pines) had a direct effect on NSC and SS contents, but no differences in starch were found (Tables 5, 6) or between both pine forests (Table 6).

Relationships among variables

The relationships in living pines between the main study variables are provided in Fig. 5. Fv/Fm showed a sigmoid relationship with WP. The maximum Fv/Fm values reached −4 MPa, but then sharply dropped to −8 MPa, with values that came close to 0 for declining trees (not included in the regression analysis). Ks and WP followed a similar trend, and gave decreasing hydraulic conductance values when WP reached −2 MPa, or lower, and showed almost no conductance when WP dropped to −8 MPa (grey points depict declining pines). Starch content showed no relationship with WP in living pines. SS was significantly related to WP, which showed that SS decreased with high drought stress levels.

Bark beetle attack

Bark beetles were detected in some forests with decay symptoms. However, the degree of the impact of bark beetles differed among the pine forests under study. Orihuela was severely affected by bark beetles, where the

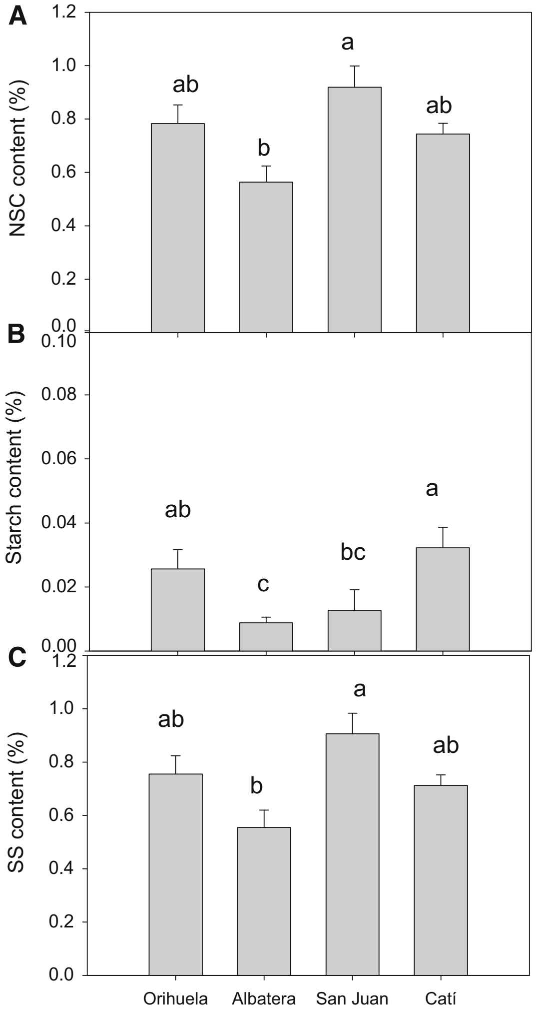

Fig. 4 Comparison (% dry matter) of **a** NSC content, **b** starch content, and **c** SS among all the forests for living pines. *Different letters* mean significant differences at $P < 0.05$ (Tukey-*B* test). Values are the mean ± SE of $n = 5$ samples at each site

values of infested pines came close to 100 % of the dead pines (Fig. 6). Conversely, the Albatera and San Juan forests were irregularly affected and obtained values of around 22 and 15 %, respectively. No attack symptoms were observed in living pines, including Catí.

Table 6 NSC, Starch and SS content (% dry matter) between living and declining pines

		Albatera	San Juan
NSC (%)	Living	0.56 ± 0.06a	0.92 ± 0.08a
	Declining	0.33 ± 0.10b	0.30 ± 0.06b
Starch (%)	Living	0.009 ± 0.002	0.013 ± 0.002
	Declining	0.010 ± 0.003	0.013 ± 0.003
SS (%)	Living	0.555 ± 0.065a	0.906 ± 0.076a
	Declining	0.315 ± 0.104b	0.296 ± 0.063b

Comparisons were made in the Albatera and San Juan forests, which were compared by two-way Anova by taking pine forests and tree condition as the main factors (see Table 5)

** $P < 0.001$; **$P < 0.01$; * $P < 0.05$. Values are the mean ± SE of $n = 4$–5 samples in each pine forest

Discussion

The results of this study reveal that ecosystems in southeast Spain were subjected to intense drought and warm conditions throughout 2014, mainly in spring and autumn. The climatic data recorded during the hydrologic year (September 2013 to August 2014) showed a reduction in precipitation of over 50 %. Temperature anomalies also reflected an especially warm year, with averaged temperatures rising by more than 1–3 °C for several months compared to the normal values. As a result of such intense dry warm conditions, many individuals in some pine populations died in the studied pine forests. Within the arid range of these forests (i.e. Orihuela forest), where precipitation was only about 60 Lm^{-2}(that is, about 24 % compared to historical records), living pines displayed WP that came close to -6 MPa, and pine mortality was over 90 %. Lower levels of drought stress were found and fewer individuals died in Albatera and San Juan. Albatera also had a lower WP, but the mortality there did not reach the same levels as in Orihuela as it was around 20 %. The San Juan forest, with a lower WP than -4 MPa, obtained a mortality rate of around 10 %. The declining pines had very negative WP values, which were estimated to be

Table 5 Statistical results for the carbohydrate dynamics (NSC, Starch and SS) of the main effects and interactions for the comparisons made between pine condition (living and declining pines) factors and pine forests (Albatera and San Juan)

Source	NSC			Starch			SS		
	MS	dof	*F*	MS	dof	*F*	MS	dof	*F*
Tree condition (TS)	0.81	1,14	29.7^{***}	0.000	1,14	0.03^{ns}	0.81	1,14	29.7^{***}
Pine forests (PF)	0.12	1,14	4.5^{ns}	0.000	1,14	1.9^{ns}	0.12	1,14	4.5^{ns}
TSx PF	0.16	1,14	5.8^{*}	0.000	1,14	0.01^{ns}	0.15	1,14	5.6^{*}

** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Values are the mean ± SE of $n = 4$–5 samples in each pine forest

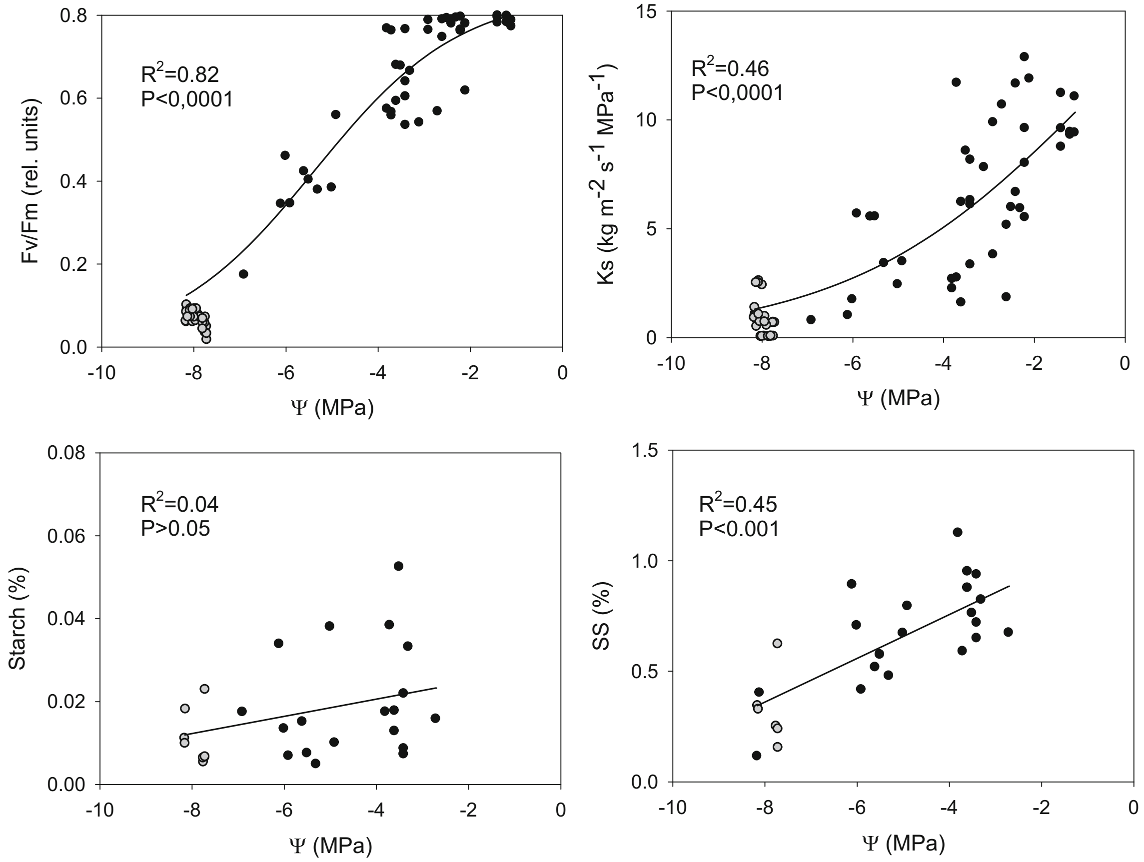

Fig. 5 Relationships among the main variables in all four pine forests. Black symbols are the values recorded in living pines; grey symbols denote declining pines. A regression analysis was carried out only in living pines

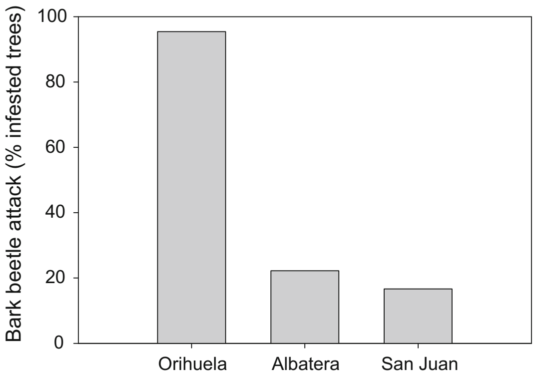

Fig. 6 Amount of dead pines (%) affected by bark beetle attacks in each forest. Data correspond to dead pines at the end of November (last field sampling date). No attacks were recorded in living pines, including the Catí forest, which presented no dead pines. $N = 20$ dead pines were sampled per pine forest

lower than −7.5 MPa, and surpassed the capacity of our pressure chamber (see Suppl. Mat.).

Drought stress also had an impact on other functional variables, such as Fv/Fm, whose values were well below 0.4 and 0.1 for living pines and declining pines, respectively. This water stress intensity also influenced Ks by causing a considerable loss of water conducting capacity due to xylem cavitation. Pines, specifically *P. halepensis*, are very sensitive to drought-induced cavitation, and a loss of hydraulic conductivity above 95 % implies very little likelihood of recovery, and thus high mortality rates (Froux et al. 2002; Brodribb and Cochard 2009). In our case, the Ks values of the living pines from the driest populations, i.e. Orihuela, were under 20 % of their maximum. This decline in Ks paralleled the water stress gradient measured in WP terms in all the forests. Hence, Albatera and San Juan showed Ks losses of about 50 %, and no decline was observed in Catí, where Ks remained high and constant, even after the autumn rains. In line with this, and according to previous studies (Brodribb and Cochard 2009), reductions in Ks due to xylem cavitation have been observed, which would be the main factor responsible for pine mortality since xylem cavitation in declining pines was higher than 80 %, and WP was lower than −7.5 MPa. Indeed, the vulnerability curves for this species, obtained from the data recorded in the field, showed severe reductions, close to 0 %, of the initial conductance at −8 MP. These findings agree with previously reported vulnerability curves for this species (Froux et al. 2002; Oliveras et al. 2003). At the end of summer, the living pines in Orihuela obtained similar values to the declining pines in San Juan or Albatera. This finding clearly indicates the impact that drought had on this population.

Several studies have reported *P. halepensis* to be a drought-avoider isohydric species that has efficient stomatal control over water use (Baquedano et al. 2008; Klein et al. 2011). This means that it can extract water quickly when it is plentiful, but it also has mechanisms that limit water use and rapidly close stomata to reduce carbon uptake in order to avoid hydraulic failure. As reported in other *Pinus* species, this behaviour under recurrent drought conditions can condition the vitality of this species in relation to carbon starvation processes (McDowell et al. 2011b). Currently, the role of whole-tree C partitioning in drought-induced tree mortality is a subject of ongoing debate (Sala et al. 2010, McDowell et al. 2011, Hartmann et al. 2013). Under acute stress conditions associated with stomatal closure, stored carbohydrates can be used in respiration, which confers more tree survival chances. Our results revealed that NSC were low, and no significant differences were observed for starch between living and declining pines. These values can be considered quite low if compared with other reported values in branches or in tree boles. Sala and Hoch (2009) reported values of 2–6 % in branches of ponderosa pines and of 1.2–2.0 % in sapwood boles. Anderegg and Anderegg (2013) published values that ranged from 2 to 8 % in branches of *Pinus edulis*. Hoch et al. (2003) also indicated similar values for *Pinus sylvestris* throughout one year. For some Mediterranean species, Rosas et al. (2013) found values that ranged from 0.5 % to 15 % in branches of *Quercus. ilex*, *Arbutus unedo* or *Phillyrea latifolia*. SS also displayed values that fell within the 0.55–0.90 % range of dry matter content, which were low if compared with other studies (Hoch et al. 2003; Anderegg and Anderegg 2013). Overall, the NSC values reported herein ranged from 0.55 to 1.0 %, which is low compared to other results reported in the branches of *Pinus sp* or for other Mediterranean species (Sala and Hoch 2009; Anderegg and Anderegg 2013; Rosas et al. 2013), or for the same species, but with intense defoliations as a result of pine processionary (Palacio et al. 2012).

NSC have been reported to quickly lower under drought conditions, especially in isohydric species (McDowell et al. 2011; Anderegg 2012). These results support the hypothesis that carbon limitation plays a key role in tree vitality and, consequently, in the tree decline and mortality observed in this study. Yet the fact that a clear relationship was lacking between starch and the WP gradient in living pines indicates that carbohydrate content was low, regardless of the level of drought that affected pines. Probably the dry conditions and high temperatures recorded throughout the year led to reserve substances being consumed. Only the SS fraction showed a significant relationship with WP, and differences between living and declining pines were found. This result was not obtained for starch content, which implies that under intense drought conditions, starch reserves are mobilised to SS to meet physiological demands. However, the amount of NSC in the present study was also low compared with other works conducted in the same tissues, which reflects that pines are affected by marked limiting conditions.

Another key point that has been reported in forest decline is the impact that pests can have from, for instance, bark beetle outbreaks or fungi attack (Hartmann et al. 2015). The results of the present study revealed that beetle attack was more important in forests that had the highest mortality rates and presented more intense dry conditions, where almost 100 % of dead trees exhibited evident signs of bark beetles. When drought conditions were not so intense (i.e. Albatera and San Juan), bark beetle attacks were unequal with infestation rates of 16–22 % on dead pines. No bark beetle attack signs were observed in living pines in any of our study forests. These results highlight the observations made in previous studies, in which drought stress reduced resin production by pines, affected the resin canal network or impaired resin exudation to produce more intense and dangerous attacks (McDowell 2011; Netherer et al. 2015). Likewise, drought conditions in pines also bring about changes in the volatile emission of terpenes or other secondary compounds, which better attract bark beetles, so more trees are affected (Gaylord et al. 2013; Raffa 2014). Netherer et al. (2015) recently reported a direct link between tree symptoms of drought and bark beetle attack. However, the irregularity of beetle infestation in our forests did not allow us to relate dead pines with infestation of some forests (i.e. Albatera and San Juan). Hence, bark beetle infestation seems to behave like opportunistic diseases, which could be related as an additional vulnerability factor under severe stress conditions as it can raise pine die-off chances.

The recovery capacity of plant species after drought events is considered a relevant factor to withstand stress conditions and to recover plant functionality. In the present study, recovery capacity was dependent on the variable considered and showed, in some cases, high levels of variability, which reflects the complexity of the physiological processes related to the water stress level reached. The maximum photochemical efficiency of PSII (Fv/Fm) completely recovered after the autumn rains, which indicates that they are well protected from stress conditions, and is in line with what other studies have pointed out for other Mediterranean species (Vilagrosa et al. 2010). WP also recovered non-lethal levels (e.g. higher than −3 MPa) after the autumn rains. However, recovery was worse in Orihuela than in Albatera and San Juan which reached the WP levels recorded in Catí. Xylem functionality recovery in each forest is probably a determining factor of tree functional recovery. In fact, xylem conductance recovery in the trees of Orihuela was significantly poorer than in the other forests, including Albatera and San Juan, which presented similar Ks values after the summer drought. At the end of summer, the living pines in Orihuela obtained similar values to the dead pines in San Juan or Albatera, which reflects the impact of drought stress on the Orihuela forest pines. In plants, xylem hydraulic conductance recovery has two possible mechanisms: xylem refilling, which typically refills embolised conduits within hours or days in angiosperms (Bucci et al. 2003; Salleo et al. 2004); xylem repair, as the growth of a new ring (Brodribb and Cochard 2009); both can be common mechanisms in *P. halepensis* (De Luis et al. 2011). Due to the torus–margo complex in conifers, whether tracheids can be refilled after widespread cavitation is still a controversial issue (Sobrado et al. 1992; Edwards et al. 1994). Recent studies have suggested that the tracheid embolism is non-reversible after drought (Brodribb and Cochard 2009), but needs to be explored for P. *halepensis* in particular. Given the time that hydraulic conductance takes to recover, Brodribb and Cochard (2009) attributed new sapwood growth in conifers to xylem functionality recovery. Although we were unable to specify whether recovery took place by refilling or new growth, we observed notable recovery in the Albatera and San Juan forests and non-statistical differences for the non-stressed pines in Catí. Conversely, recovery in the Orihuela forest occurred, but was significantly poorer. This means that the water transport of the pines there was quite limited despite the partial WP recovery.

Conclusions

This study hypothesised that lack of rain during the current year may favour a decline in pines, but mortality would be promoted mainly by bark beetle attack. Our results show a direct association between pine mortality and severe drought conditions. Such conditions produced high levels of xylem cavitation, which promoted pine mortality. Our results also indicate that bark beetles did not seem to be directly involved in mortality, at least not under less intense drought conditions (e.g. San Juan and Albatera) since dead pines showed moderate signs of beetle attack (10–20 %). Therefore, bark beetle attacks should be considered an additional vulnerability factor to raise pine mortality chances.

The results also suggest that hydraulic failure and carbon starvation are likely interrelated, which makes it difficult to separate both mechanisms. However, it is still unknown if a low carbohydrate content can be considered a determinant factor since the results presented herein reveal that the Catí forest had a low NSC content, but no declining trees.

Our results also reflect that semiarid climates entail major limiting conditions for *Pinus halepensis,* which would be in a high risk of future mortality events due to climate variability.

Author contribution statement R. García de la Serrana and A.Vilagrosa: Both authors participated equally in the study designing, sampling, analysing and writing the manuscript. J. A Alloza was in charge of the climatic database, and of the analysis and discussion of climatic trends in the study plots.

Acknowledgments We thank JA. Valiente for supplying the CEAM weather database and JM. Torres for his suggestions to improve the manuscript. This work has been carried out, thanks to Projects SURVIVE (CGL-2011-30531-CO2-02) and GRACCIE (CTM2014-59111-REDC, RED CONSOLIDER-INGENIO 2014 Programme), funded by the Spanish Government, and the PROMETEO programme (DESESTRES 2014/038), funded by Generalitat Valenciana (Regional Valencian Government). R.García de la Serrana is grateful for the Geronimo Forteza grant (FPA/2014/126), funded by Generalitat Valenciana to the SURVIVE project. CEAM is supported by the Generalitat Valenciana.

Compliance with the ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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