

Drought-induced xylem cavitation and hydraulic deterioration: risk factors for urban trees under climate change?

Tadeja Savi, Stefano Bertuzzi, Salvatore Branca, Mauro Tretiach and Andrea Nardini

Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, Trieste 34127, Italy

Author for correspondence:

Andrea Nardini

Tel: +39 040 5583890

Email: nardini@units.it

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Summary

- Urban trees help towns to cope with climate warming by cooling both air and surfaces. The challenges imposed by the urban environment, with special reference to low water availability due to the presence of extensive pavements, result in high rates of mortality of street trees, that can be increased by climatic extremes.

- We investigated the water relations and xylem hydraulic safety/efficiency of *Quercus ilex* trees growing at urban sites with different percentages of surrounding impervious pavements. Seasonal changes of plant water potential and gas exchange, vulnerability to cavitation and embolism level, and morpho-anatomical traits were measured.

- We found patterns of increasing water stress and vulnerability to drought at increasing percentages of impervious pavement cover, with a consequent reduction in gas exchange rates, decreased safety margins toward embolism development, and increased vulnerability to cavitation, suggesting the occurrence of stress-induced hydraulic deterioration.

- The amount of impermeable surface and chronic exposure to water stress influence the site-specific risk of drought-induced dieback of urban trees under extreme drought. Besides providing directions for management of green spaces in towns, our data suggest that xylem hydraulics is key to a full understanding of the responses of urban trees to global change.

Introduction

Ongoing climate changes are increasing the frequency of drought and heatwaves in several areas of the globe (Prudhomme *et al.*, 2014), and urbanized areas will be likely hotspots of temperature rise, as a consequence of the 'urban heat island' effect (Oleson *et al.*, 2011). Increasing temperatures in towns are predicted to imply significant economic and social costs over coming decades (Scheraga & Grambsch, 1998; Luber & McGeehin, 2008), thus calling for the adoption of mitigation and adaptation strategies by local municipalities and national governments.

Urban trees represent effective tools to improve urban climate (Bowler *et al.*, 2010; Sung, 2013), as they effectively cool down air and surfaces (Armson *et al.*, 2012; Sung, 2013) through shading effects and evaporative processes (Pataki *et al.*, 2011). However, the effectiveness of ecosystem services provided by trees can be impaired by canopy dieback induced by abiotic (Vilagrosa *et al.*, 2003) and biotic stress factors (Nardini *et al.*, 2004; Meineke *et al.*, 2013), or as a consequence of prolonged stomatal closure induced by water stress (Bush *et al.*, 2008; Litvak *et al.*, 2012). Hence, the success of urban forestry as a mitigation strategy against climate warming depends on the health status of trees, which in turn has important consequences for the health of people living in towns (Villeneuve *et al.*, 2012; Donovan *et al.*, 2013).

Over the last decades, episodes of tree decline triggered by drought and heatwaves have been reported for different

ecosystems (Allen *et al.*, 2010; Michaelian *et al.*, 2011; Peng *et al.*, 2011). The physiological bases of drought-induced tree decline are still unresolved and likely involve at least three functional levels: water transport, carbon metabolism and plant responses to biotic agents (McDowell *et al.*, 2011, 2013). The drought-induced reduction of plant water potential can lead to massive xylem cavitation and impairment of root-to-leaf water transport, thus leading to plant desiccation (Breshears *et al.*, 2013; Nardini *et al.*, 2013; Mitchell *et al.*, 2014). In turn, prolonged stomatal closure caused by reduced water transport capacity, or addressed at preventing xylem pressure drop (Salleo *et al.*, 2000), can lead to depletion of nonstructural carbohydrate reserves (Adams *et al.*, 2013; Poyatos *et al.*, 2013; Sevanto *et al.*, 2014). Finally, carbon starvation might lead to impairment of plants' defense strategies against biotic agents (Rivas-Ubach *et al.*, 2014), favoring heavy attacks by different pests (McDowell, 2011; Anderegg & Callaway, 2012; Gaylord *et al.*, 2013). Whatever the exact mechanism leading to tree decline, there is general consensus around the crucial role played by vulnerability to xylem cavitation in driving plants' responses to extreme climatic events (Rice *et al.*, 2004; Brodrribb & Cochard, 2009; Choat *et al.*, 2012). Hence, it is not surprising that several studies have investigated the correlations between tree hydraulics and drought performance in natural habitats (Pratt *et al.*, 2008; Hoffmann *et al.*, 2011; Nardini *et al.*, 2013). However, the hydraulics of urban trees has only rarely been considered as a possible

vulnerability factor modulating the risk of canopy dieback under environmental stress (Bush *et al.*, 2008; Litvak *et al.*, 2012).

The challenges imposed by the urban environment on plant functioning result in high rates of drought-induced mortality of street trees (Nowak *et al.*, 2004; Roman *et al.*, 2014), that can be exacerbated by climatic extremes (Helama *et al.*, 2012; May *et al.*, 2013). Urban trees can be exposed to significant water stress because of high air temperatures and reduced water availability (Gillner *et al.*, 2013). In particular, water supply to urban trees is complicated by soil compaction (Yang & Zhang, 2011) and by extensive impervious surfaces preventing or delaying recharge of soil water content following rainfall events (Morgenroth *et al.*, 2013). These factors can also reduce root growth and vitality via mechanical effects (Day *et al.*, 2010) or by altering gas diffusion through the rhizosphere (Viswanathan *et al.*, 2011) with predictable chronic water restrictions for trees growing in areas with compacted and/or impervious soils (Ugolini *et al.*, 2012). On the one hand, chronic exposure to moderate water stress might drive acclimation responses of the tree hydraulic system in terms of efficiency and safety (Awad *et al.*, 2010; Wortemann *et al.*, 2011), making urban trees less susceptible to extreme events. On the other hand, long-term water stress experienced by trees growing in less favorable spots in terms of water availability, might actually drive the progressive hydraulic deterioration of the xylem system via 'cavitation fatigue' (Hacke *et al.*, 2001) processes, as recently shown for poplar (Anderegg *et al.*, 2013) and ash (Nardini *et al.*, 2014), thus increasing the vulnerability of trees to successive drought events.

We specifically addressed these questions by comparing the water relations and xylem hydraulic safety/efficiency of *Quercus ilex* (holm oak) trees growing in urban sites characterized by different levels of impermeability of surrounding soil. We specifically tested the following hypotheses: trees growing in areas characterized by extensive impervious surfaces experience more intense seasonal water stress than those growing in areas with permeable surfaces; water stress translates into higher levels of embolism and reduced gas exchange rates; chronic water stress leads to increased vulnerability to xylem cavitation and hydraulic deterioration (Anderegg *et al.*, 2013) in trees growing in areas with extensive impervious surfaces.

Materials and Methods

Study sites and plant material

Experiments were conducted in the urban area of Trieste, Italy, a middle-sized town (210 000 inhabitants) on the Adriatic coast, and spreading over a substrate of Eocene Flysch deposits (Onofri, 1982). The climate of Trieste is transitional between the Mediterranean and Central European types, with equinoctial rainfalls, cold winters and relatively dry periods in December–February and July–August (Codogno & Furlanetto, 2004). The mean annual temperature averages 14.4°C, and annual rainfall totals 1065 mm (<http://www.meteo.fvg.it>). Approximately 4% of the urban zone is covered by green areas in which Mediterranean tree species are often used as ornamental plants. The species

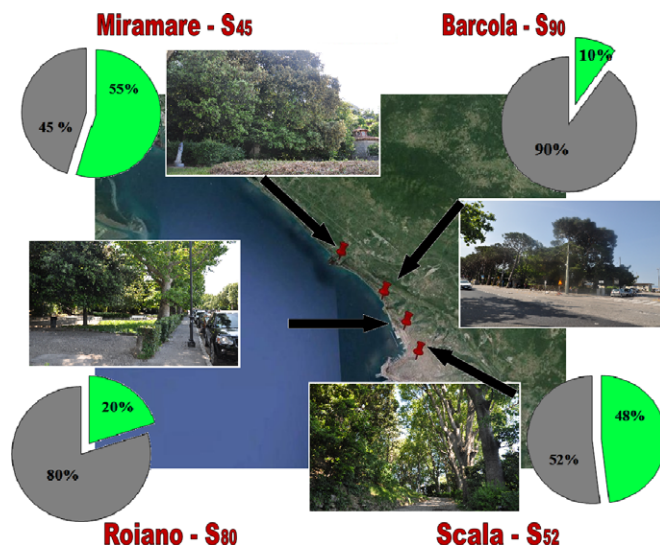


Fig. 1 Location of the four experimental sites along the coastline of the Municipality of Trieste (northeastern Italy). The percentages of surrounding soil surface cover with pervious (green) or impervious pavement (gray) are reported (see the Materials and Methods section for details).

selected for the study is *Quercus ilex* L. (holm oak) due to its widespread presence in Trieste and availability of previous studies focused on its water relations (Tretiach, 1993; Nardini *et al.*, 2000). Four sites were selected along a NW–SE transect parallel to the coastal line (Fig. 1), on the basis of the presence of at least three adult individuals of *Q. ilex* and different percentages of soil coverage with impervious substrates. The percentage of paved surface at each site was quantified in a circular area centered on experimental trees and with a radius of 25 m, for a total surface of *c.* 2000 m². Spatial analysis was performed on Google Earth images (<http://www.google.com/earth>) processed using ImageJ (<http://rsbweb.nih.gov/ij/>), and confirmed by direct field surveys. At all sites, unpaved soil was covered by grass or small shrubs and was apparently not subjected to significant compaction.

All selected trees were > 20-yr old, with a height between 6 and 10 m. Experimental measurements were performed on three individuals per site between June and September 2013. Climatic data (air temperature and relative humidity) were continuously recorded (at 30-min intervals) throughout the study period by EL-USB-2 dataloggers (Laskar Electronics Inc., Salisbury, UK) positioned at a height of 3 m on the north-facing side of the main trunks of two individuals per site. The study period was characterized by abundant rainfall in May–June (202 mm) and a dry period in July–August (55 mm), interrupted in early September (10 d before the last seasonal measurements) by late-summer thunderstorms accumulating a total rainfall of 97 mm.

Measurements of leaf gas exchange rates and plant water status

The leaf conductance to water vapor and water potential were measured on a monthly basis on fully expanded current-year leaves, sampled from the outer portion of the south-facing canopy. Pre-dawn leaf water potential (Ψ_{pd}) was measured between

05:00 and 06:00 h (solar time). Two leaves per individual (six leaves per site) were collected and immediately wrapped in clingfilm, stored in a refrigerated bag and transported to the laboratory within 30 min. Water potential was measured using a pressure chamber (mod. 1505D; PMS Instrument Company, Albany, OR, USA). The sampling was repeated on the same day between 11:00 and 13:00 h, to estimate minimum daily leaf water potential (Ψ_{\min}). Minimum xylem water potential (Ψ_{xyl}) was also estimated by measuring the water potential of leaves that had been covered with clingfilm and aluminum foil at pre-dawn, to allow equilibration of leaf water potential with xylem pressures. On the day following Ψ measurements, leaf conductance to water vapor (g_L) was measured between 11:00 and 13:00 h. Measurements were performed on two leaves per individual (six leaves per site) using a steady-state porometer (SC-1; Decagon Devices Inc., Pullman, WA, USA). All measurements were performed on clear sunny days with PPFD ranging between 700 and 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Measurements of photosynthetic efficiency

On the same dates and times when g_L was measured, the photosynthetic efficiency was estimated by Chlorophyll *a* Fluorescence (Chl_aF) emission measurements, performed on three leaves per individual (nine leaves per site) selected as detailed above and darkened 20 min before measurements. Measurements were done with a portable fluorometer (Handy Pea; Hansatech, Norfolk, UK), and F_v/F_m was calculated as a proxy for quantum yield of *PSII* (Maxwell & Johnson, 2000).

Measurements of xylem vulnerability to cavitation and percentage loss of stem hydraulic conductivity

In order to assess site-specific tree vulnerability to drought-induced xylem cavitation, vulnerability curves (VCs) were measured for 2-yr-old stems using the bench dehydration technique (Cochard *et al.*, 2013). Measurements were performed in July 2013, after the May–June rainy period and before the onset of substantial water stress. Five-year-old branches (randomly selected from the three individuals in each site) were cut underwater in the field between 07:00 and 08:00 h. Branches were transported to the laboratory and kept with their cut ends immersed in water and with foliage enclosed in a plastic bag for 24 h. This procedure favored full branch rehydration and refilling of eventually embolized conduits (Trifilò *et al.*, 2014a). Branches were removed from water and dehydrated on the bench at laboratory irradiance, temperatures ranging between 20 and 22°C, and relative humidity averaging 45%. At 60–90-min intervals, Ψ_{xyl} was measured in wrapped leaves. Hydraulic measurements were performed on 20–22 samples at different Ψ_{xyl} (between 0 and -3.5 MPa). Two-year-old stems were recut underwater to a length of 5–7 cm, connected to an hydraulic apparatus (Xyl'Em – Xylem Embolism Meter; Bronkhorst, Montigny-les-Cormeilles, France) and perfused with a commercial mineral water containing several elements in ionic form and with K^+ adjusted to 10 mM (Nardini *et al.*, 2011). The solution was initially perfused through stems at

a pressure (P) of 8 kPa until the flow (F) became stable (within 10–12 min). Stems were then flushed at $P = 0.2$ MPa for 15 min to remove embolism and flow was re-measured at $P = 8$ kPa. Stem hydraulic conductivity (K) was computed as $(F/P) \times l$ (l , stem length). The initial K (K_i) and the value measured after embolism removal (K_{\max}) were used to estimate the percentage loss of stem hydraulic conductivity (PLC) as:

$$\text{PLC} = 1 - (K_i/K_{\max}) \times 100 \quad (1)$$

In order to assess the impact of seasonal water stress on xylem functioning, midday PLC values experienced by plants were measured on a monthly basis, in the same weeks when Ψ and g_L measurements were performed. Two-year-old stems (two per individual, six stems per site) were cut underwater in the field between 11:00 and 13:00 h, and transported to the laboratory within 30 min, while remaining immersed in water. Samples were re-cut underwater to a length of 5–7 cm and their PLC was measured as described above. Upon sampling in the field, Ψ_{xyl} was measured as described above.

In August, when maximum seasonal PLC was recorded at all sites (see the Results section), all leaves inserted distally to the stem segments used for hydraulic analysis were detached, and total surface area (A_{leaf}) was measured by scanning leaves and analysing images with ImageJ. Leaf specific hydraulic conductivity of stems (LSC) was calculated as K/A_{leaf} and using both K_i and K_{\max} values, thus getting LSC_i and LSC_{\max} .

Morpho-anatomical measurements

Wood density and vessel size were measured for a sub-set of stems used for hydraulic measurements (15 samples per site). Two-centimeter-long samples were fully rehydrated overnight while immersed in distilled water. After bark removal, their fresh volume was measured by water displacement (Hughes, 2005). Samples were oven-dried for 24 h at 70°C to determine their dry mass. Wood density (δ_w) was calculated as dry mass/fresh volume (Marksteijn *et al.*, 2011). Stem cross-sections (three per individual, nine samples per site) were obtained using a microtome and immediately observed under a light microscope. Images were acquired using a digital camera and analyzed with ImageJ. Xylem conduit diameters were measured on at least 20–30 randomly selected elements per section.

Statistics

Statistical analysis was performed using SigmaStat v2.0 (Systat, San Jose, CA, USA). The significance of differences was tested using one-way ANOVA followed by Tukey's *post-hoc* comparisons. The significance of correlations was tested using the Pearson product-moment coefficient.

Results

The four study sites differed in terms of percentage paved soil surface. In the Miramare (S_{45}) and Scala (S_{52}) sites, < 50% of the

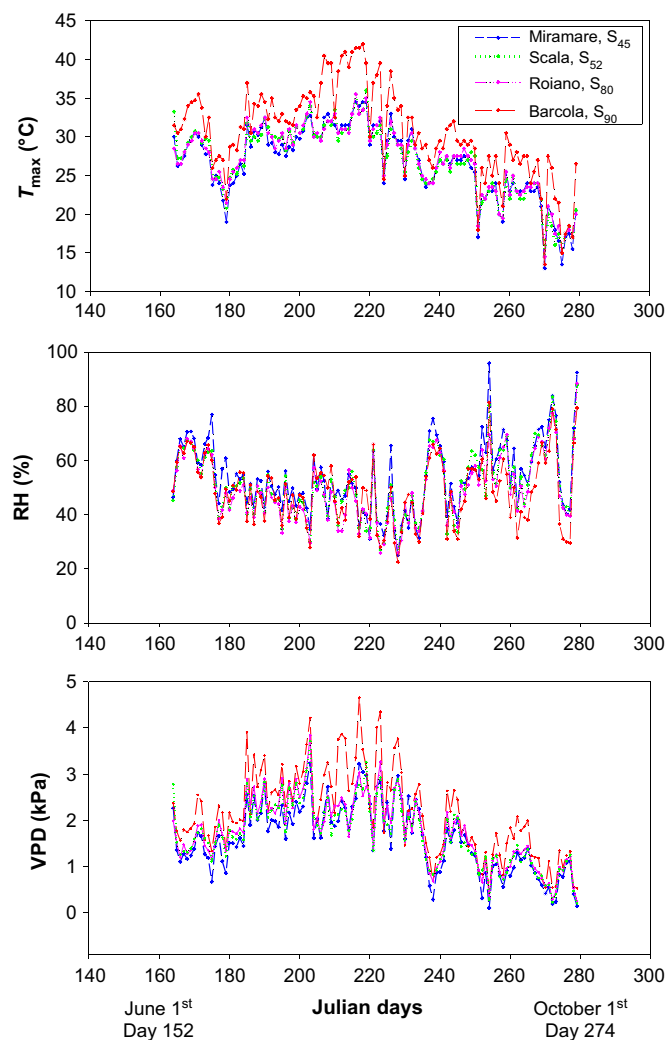


Fig. 2 Seasonal trend of daily maximum air temperatures (T_{\max}), minimum relative humidity (RH) and vapor pressure deficit (VPD) as measured at the four study sites. Each point represents the mean of values recorded by two sensors located at each site (see Materials and Methods section for details).

soil surface was impermeable, increasing to 80% and 90% in Roiano (S_{80}) and Barcola (S_{90}), respectively (Fig. 1). In addition, sites S_{80} and S_{90} had higher air temperatures and lower relative humidity than the other sites (Fig. 2). For example, in early August air temperatures in S_{90} peaked at 40°C, but were 7–9°C lower in S_{45} and S_{52} . The maximum daily vapor pressure deficit (VPD) was 0.5–2 kPa higher in S_{90} and S_{80} than in S_{45} and S_{52} ; the mean of maximum daily VPD values recorded from early June to mid-September were 1.59 ± 0.74 , 1.70 ± 0.71 , 1.75 ± 0.73 and 2.15 ± 0.93 kPa in S_{45} , S_{52} , S_{80} and S_{90} , respectively.

In June, after a prolonged rainy period, g_L averaged $450 \text{ mmol s}^{-1} \text{ m}^{-2}$, with peaks up to $700 \text{ mmol s}^{-1} \text{ m}^{-2}$ in S_{45} (Fig. 3). These values remained substantially stable in July, at the early onset of summer drought, but decreased significantly in August, when g_L averaged $320 \text{ mmol s}^{-1} \text{ m}^{-2}$ across sites. In September, following late-summer rainfall, g_L recovered in the sites

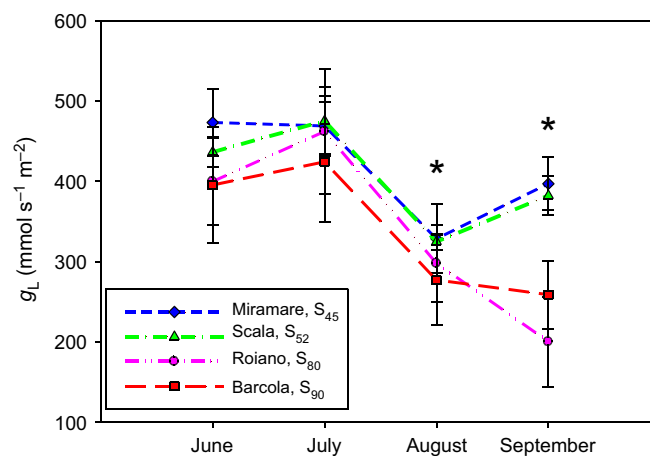


Fig. 3 Seasonal changes of leaf conductance to water vapor (g_L) as measured in *Quercus ilex* trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Means are reported \pm SEM. Significant differences between sites (one-way ANOVA): *, $P < 0.05$.

with the lowest percentage of impervious surface (S_{45} and S_{52}), where it returned to 397 ± 33 and $382 \pm 24 \text{ mmol s}^{-1} \text{ m}^{-2}$, respectively. However, a further g_L drop was observed in S_{80} and S_{90} (200 ± 23 and $259 \pm 43 \text{ mmol s}^{-1} \text{ m}^{-2}$, respectively).

In June, Ψ_{pd} ranged between -0.1 and -0.6 MPa in S_{45} and S_{90} , respectively (Fig. 4). A slight seasonal Ψ_{pd} decrease was observed in all study sites, but this was much more abrupt in S_{90} in August, when Ψ_{pd} dropped to *c.* -1.2 MPa, whereas in the other sites it ranged between -0.4 and -0.6 MPa. Both Ψ_{\min} and Ψ_{xyl} were found to be significantly higher throughout the season in S_{45} and S_{52} than in S_{80} and S_{90} . Absolute minimum values of Ψ_{\min} and Ψ_{xyl} were recorded in S_{90} in August (-2.5 ± 0.3 and -2.0 ± 0.3 MPa, respectively), whereas trees growing in S_{52} experienced the lowest level of water stress, with Ψ_{\min} and Ψ_{xyl} averaging -1.0 ± 0.2 and -0.5 ± 0.1 MPa, respectively (Fig. 4). Leaf water potential increased after the September rainfall, but the magnitude of the recovery varied among sites. As an example, in S_{45} Ψ_{xyl} increased from -1.6 MPa in August to -0.7 MPa in September, whereas in S_{90} Ψ_{xyl} changed only from -2.0 to -1.8 MPa in the same time interval. A clear correlation emerged between g_L and Ψ_{\min} , both within sites on a seasonal basis and across sites in each study period. Fig. 5 reports the correlation between these parameters across all sites and study periods.

Table 1 reports values of F_v/F_m measured in August, at the peak of seasonal water stress. Significant differences were recorded among sites, with S_{45} and S_{52} displaying the highest F_v/F_m (*c.* 0.82) and progressively lower values recorded in S_{80} (*c.* 0.79) and S_{90} (*c.* 0.77). Slightly significant differences were observed in terms of wood density and xylem conduit dimensions (Table 1), but no specific trend could be observed in relation to the percentage of impervious surface surrounding trees, nor to the level of water stress suffered by individuals.

Measurements of seasonal PLC progression revealed marked differences between the study sites (Fig. 6a). Embolism levels were constantly low throughout the season in S_{45} and S_{52} , where

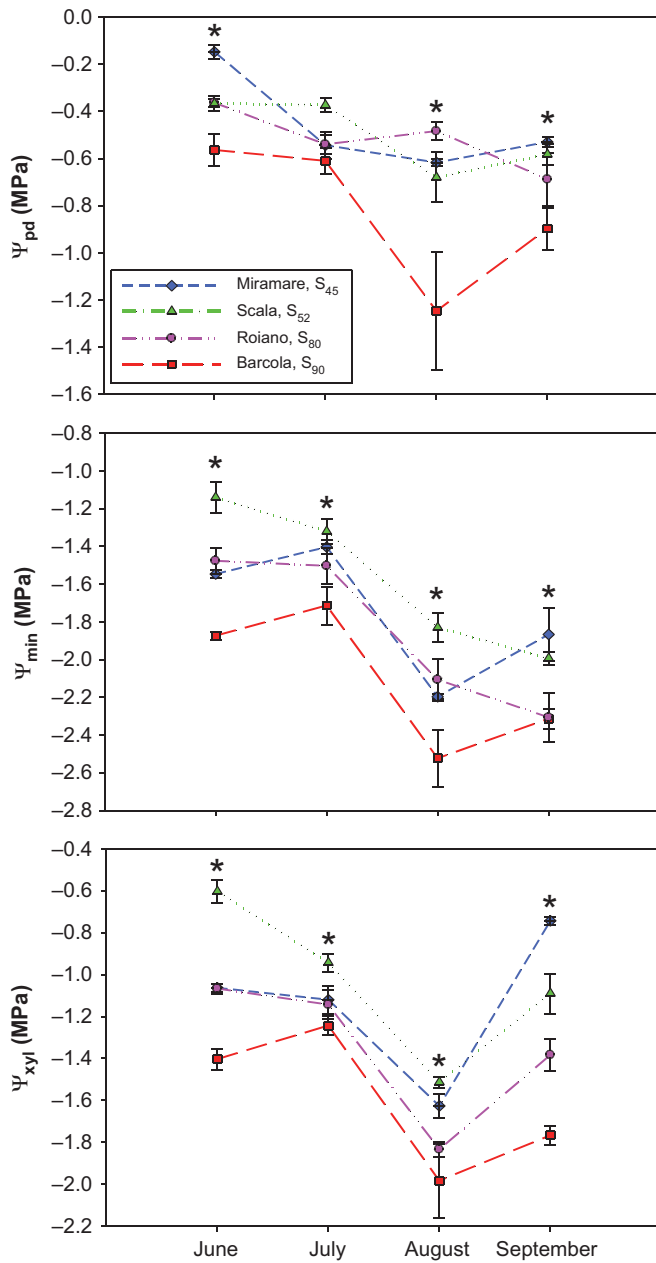


Fig. 4 Seasonal changes of predawn leaf water potential (Ψ_{pd}), and minimum daily leaf (Ψ_{min}) and xylem (Ψ_{xyl}) water potential as measured in *Quercus ilex* trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Means are reported \pm SEM. Significant differences between sites (one-way ANOVA): *, $P < 0.05$.

PLC ranged between 25% and 40%. In S_{80} , low PLC (*c.* 20%) was recorded in May, but this value sharply increased upon onset of drought stress, reaching values as high as 70% in August. Embolism was only partially reversed in September, when PLC decreased to *c.* 50%. In S_{90} , PLC was 55% in June, already, and further increased to 70% in August. No recovery of PLC following late-summer rains was observed at this site.

In August, LSC_i was similar across individuals (Fig. 6b), despite marked differences in terms of PLC. When the same

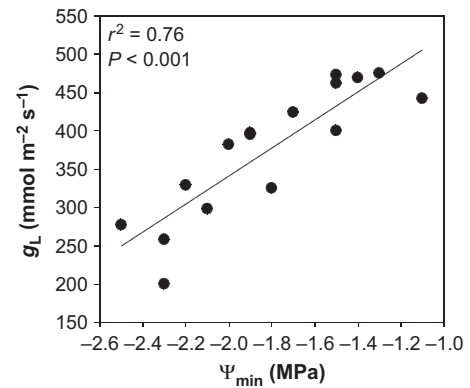


Fig. 5 Relationship between leaf conductance to water vapor (g_L) and minimum daily leaf water potential (Ψ_{min}) as measured throughout the season (June–September) in *Quercus ilex* trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Each point represents the mean value \pm SD. The regression line together with r^2 and P values is also reported.

Table 1 Wood density (δ_w), xylem conduit diameter (D_v) and chlorophyll fluorescence (F_v/F_m) as measured in *Quercus ilex* trees growing at four different urban sites with different percentages of impermeable surface cover (see Fig. 1)

	δ_w ($g\ cm^{-3}$)	D_v (mm)	F_v/F_m
Miramare (S_{45})	$0.82 \pm 0.02\ ab$	$33.5 \pm 5.1\ ab$	$0.820 \pm 0.008\ a$
Scala (S_{52})	$0.77 \pm 0.05\ a$	$37.1 \pm 7.0\ a$	$0.816 \pm 0.007\ a$
Roiano (S_{80})	$0.87 \pm 0.03\ b$	$33.4 \pm 4.8\ ab$	$0.794 \pm 0.015\ ab$
Barcola (S_{90})	$0.84 \pm 0.06\ ab$	$32.3 \pm 5.0\ b$	$0.765 \pm 0.033\ b$

Means are reported \pm SD. Different letters indicate statistically significant differences between sites ($P < 0.05$).

parameter was calculated on the basis of maximum stem hydraulic conductivity measured after embolism removal, very similar values were recorded at S_{45} , S_{52} and S_{80} , but at S_{90} the LSC_{max} was significantly higher. This trend was driven by differences in total leaf surface area supplied by 2-yr-old stems. In fact, A_{leaf} was similar in S_{45} , S_{52} and S_{80} with values of 213 ± 38 , 232 ± 56 and $275 \pm 66\ cm^2$, respectively. Significantly lower A_{leaf} was recorded in S_{90} ($93 \pm 16\ cm^2$).

Figure 7 shows vulnerability curves for trees growing at the four sites, as measured in the laboratory (black circles); the PLC measured in the field throughout the season is also plotted vs the corresponding Ψ_{xyl} measured *in situ* (open circles). Field-measured PLC values were well within the range of laboratory measurements. At all sites, VCs had a sigmoidal shape, and from the regression curves the values of Ψ_{xyl} inducing 50% loss of hydraulic conductance (Ψ_{50}) were calculated. These were lower in S_{45} and S_{52} (-1.61 and -1.54 MPa, respectively), than in S_{80} and S_{90} (-1.37 and -1.28 MPa, respectively). In order to test the statistical significance of site-specific differences in terms of vulnerability to cavitation, the average PLC induced by Ψ_{xyl} values between -1.2 and -1.7 MPa was calculated (Fig. 8). Although mean Ψ_{xyl} calculated in this range was not statistically different across sites, PLC was found to be significantly higher in samples

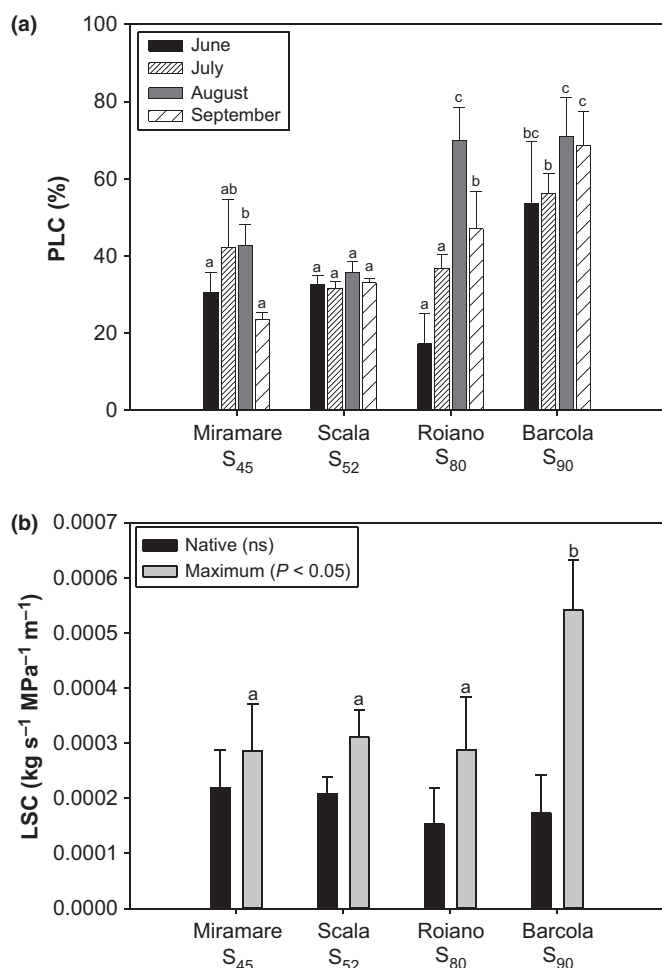


Fig. 6 (a) Seasonal changes of percentage loss of hydraulic conductivity (PLC) as measured in 2-yr-old stems sampled from *Quercus ilex* L. trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Mean values are reported \pm SD. (b) Leaf specific stem hydraulic conductivity (LSC) as measured in 2-yr-old stems sampled in August 2013. Both native values (black columns) and maximum values (gray columns) measured after complete embolism removal are reported. Mean values are reported \pm SD. Different letters indicate statistically significant differences ($P < 0.05$). ns, not significant.

from S₈₀ and S₉₀ than in those from S₄₅ and S₅₂. On the basis of Ψ_{50} and minimum seasonal Ψ_{xy1} values, the safety margin toward massive cavitation was calculated for trees growing at the different sites (Choat *et al.*, 2012). This was found to be slightly positive in S₄₅ and S₅₂ (+0.01 and +0.04 MPa, respectively), but negative in S₈₀ (−0.43 MPa) and S₉₀ (−0.72 MPa).

Across sites, VPD was not significantly correlated with the percentage of impermeable surface ($P = 0.15$). Also, no significant correlation was observed between g_L and VPD when single measurement periods or the whole dataset were considered, with the exception of a significant inverse relationship observed in July ($r = -0.97$, $P = 0.03$). By contrast, g_L was inversely correlated to the percentage of impervious pavement in June, August and September ($P = 0.02$ – 0.03), but not in July. PLC was not correlated to VPD across sites, nor within each site on a seasonal basis. By contrast, significant correlations between the amount of xylem

embolism and the percentage of impervious pavement were observed across sites in August ($r = 0.94$, $P = 0.05$) and September ($r = 0.96$, $P = 0.04$). In September Ψ_{pd} was found to decrease with increasing percentage of impervious surface (Fig. 9a). However, PLC showed a positive correlation with the impermeability level (Fig. 9b). Vulnerability to cavitation was positively correlated with the percentage of impervious surface, as progressively less negative Ψ_{50} values were recorded at increasing percentage of impermeable pavement (Fig. 9c). Finally, safety margins were found to linearly decrease as a function of the impermeability level across sites (Fig. 9d).

Discussion

A comparison of the water relations and hydraulics of holm oak trees growing in four urban sites revealed clear patterns of increasing water stress exposure and vulnerability to drought at increasing percentages of impermeable soil coverage. The consequent reduction in stomatal aperture, increase of embolism and vulnerability to cavitation, are all factors portending potential risks of dieback under future climate scenarios for trees growing in urban areas.

Plants growing in urban sites with largely paved surfaces experienced significantly higher levels of water stress than those growing in largely unpaved urban sites. Plant water status measured in early summer, following abundant and prolonged rainfall, was overall similar across sites. However, large differences were recorded in August, when Ψ_{pd} , Ψ_{min} and Ψ_{xy1} were more negative in sites with higher percentages of impervious surface (Figs 4, 9a). These differences might have been caused by higher evaporative demand (Chen *et al.*, 2011; Pataki *et al.*, 2011) and/or by reduced water supply. Indeed, VPD was higher at the extensively paved sites, but g_L was apparently not strictly related to this parameter (see the Results section). Impermeable surfaces have been reported to prevent or delay rainfall infiltration in the soil (Morgenroth *et al.*, 2013), reducing the amount of water available to plants. Restricted water availability in paved sites would be confirmed by recorded differences in terms of Ψ_{pd} , that is assumed to reflect soil water potential as far as nocturnal transpiration is low (Kavanagh *et al.*, 2007). In particular, the lack of recovery of plant water potential following late-summer rains in the extensively paved sites (Fig. 4) supports the existence of water supply limitations. Moreover, impervious surfaces may limit not only water penetration in the soil, but also soil–atmosphere gas exchange, thus creating conditions restricting root growth (Day *et al.*, 2010; Viswanathan *et al.*, 2011) and metabolism with impacts on root hydraulics (Nardini *et al.*, 1998).

The water potential drop during the summer drought translated into a decrease of g_L at the study sites, but this was larger at extensively paved sites. Moreover, at these sites stomatal aperture was not restored following late summer rains, despite the large decrease in VPD, whereas at the other sites g_L returned to pre-stress values (Fig. 3). As a consequence, the time interval available for net photosynthesis and carbon gain was significantly shortened at those sites with impermeable surfaces. Fluorescence analysis suggested the occurrence of drought-induced

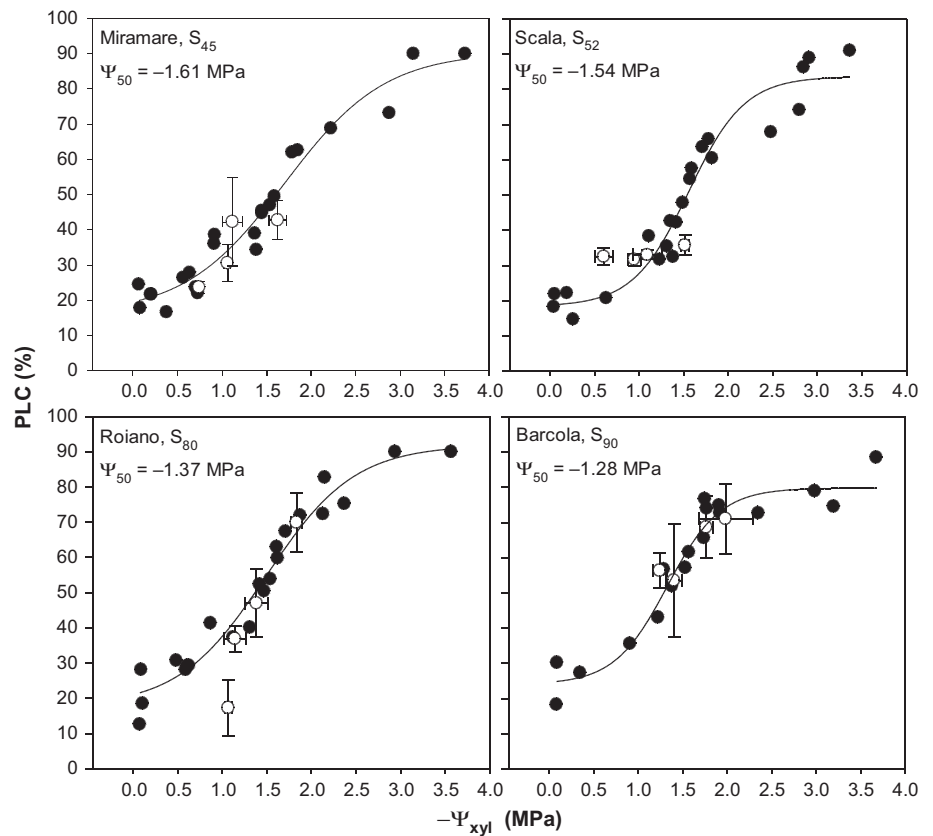


Fig. 7 Vulnerability curves reporting the relationship between percentage loss of hydraulic conductivity (PLC) of 2-yr-old stems, as measured at progressively lower xylem water potential (Ψ_{xyl}). Stems were sampled from *Quercus ilex* trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Closed circles, experimental points measured in the laboratory during bench-dehydration of branches sampled in the field; open circles, mean values (\pm SD) recorded in the field from June to September (see also Figs 4, 6). The sigmoidal regression is reported together with the calculated Ψ_{xyl} value inducing 50% loss of hydraulic conductivity (Ψ_{50}).

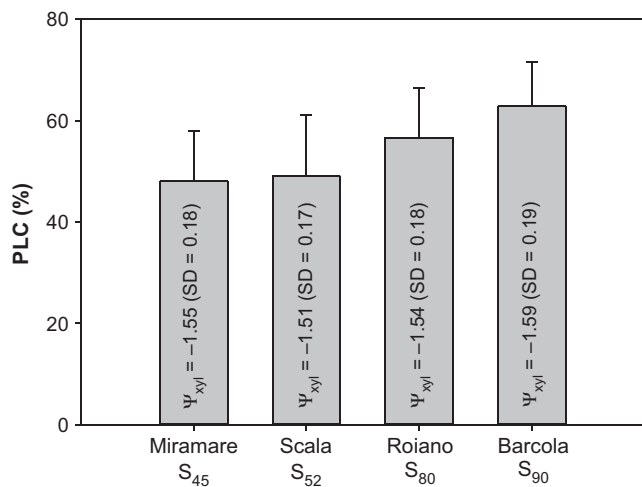


Fig. 8 Percentage loss of hydraulic conductivity (PLC) of 2-yr-old stems, as measured at a mean xylem water potential (Ψ_{xyl}) of -1.5 MPa. Stems were sampled from *Quercus ilex* trees growing at four experimental sites with different percentages of soil surface pervious or impervious pavement cover (see Fig. 1). Means are reported \pm SD. Values recorded in Roiano and Barcola are significantly higher than those of Miramare and Scala ($P < 0.05$).

impairment of *PSII* in trees growing at sites with largely impermeable surfaces, indicating that these plants also suffered non-stomatal limitations of photosynthesis (Flexas *et al.*, 2014). Because photosynthesis limitations lead to progressive depletion of nonstructural carbohydrates (Sevanto *et al.*, 2014), urban trees

exposed to chronic water stress might also become more susceptible to other abiotic or biotic stress factors (Galvez *et al.*, 2013; Gaylord *et al.*, 2013; Meineke *et al.*, 2013).

Xylem water is subjected to negative pressure, and hence it is prone to cavitation, but the likelihood of massive xylem cavitation and embolism increases at progressively decreasing Ψ_{xyl} values, according to species-specific vulnerabilities (Choat *et al.*, 2012). Xylem cavitation and embolism result in a reduction of plant hydraulic conductance, which reduces gas exchange rates and photosynthetic productivity, and also exposes plants to the risk of hydraulic failure and desiccation (Trifilò *et al.*, 2014b). In the present study, the lower Ψ_{xyl} of trees growing at sites with highly impermeable surfaces translated into higher stem PLC, especially in August and September. At the peak of summer drought (Figs 6, 9b), PLC reached values as high as 70%, close to the limit that has been reported to be critical for woody angiosperms (Nardini *et al.*, 2013). Moreover, at the site with the highest percentage of impervious surface, PLC did not recover even after late-summer rains, suggesting that plants growing in urban sites with paved soils experience a long-lasting risk of hydraulic failure.

Previous studies have reported acclimation of vulnerability to cavitation of some tree species, with Ψ_{50} values shifting toward lower values in response to moderate water stress (Beikircher & Mayr, 2009; Awad *et al.*, 2010; Wortemann *et al.*, 2011). In the present study, however, vulnerability analysis revealed that individuals exposed to more intense water stress were more, and not less, vulnerable to drought-induced cavitation, as revealed by

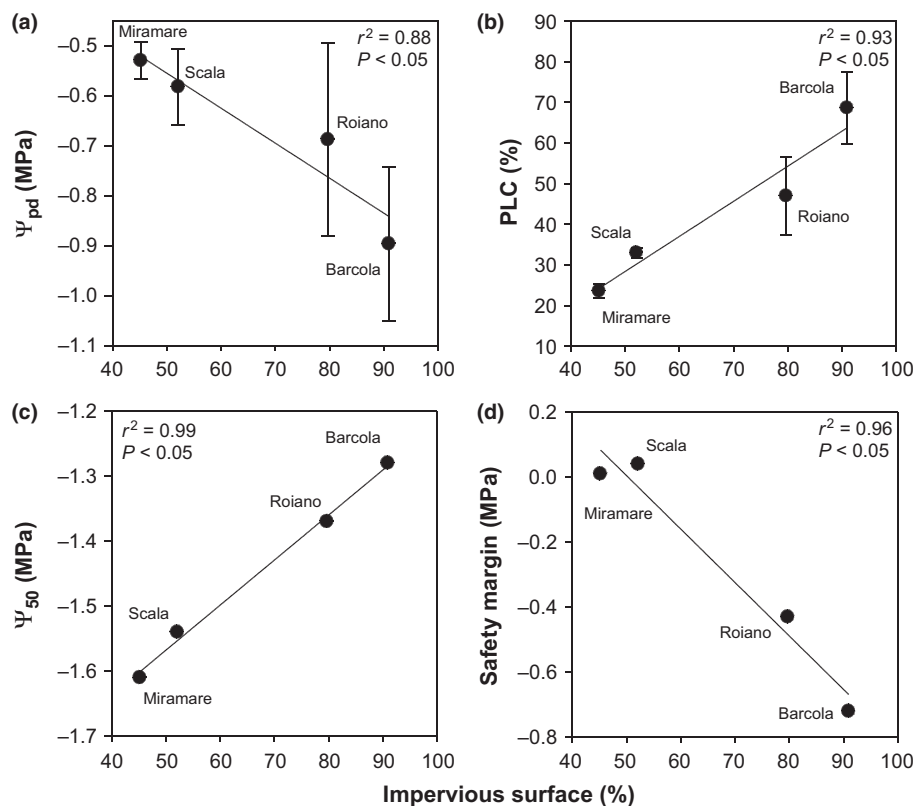


Fig. 9 Correlations between percentages of soil surface covered by impervious pavement and (a) predawn leaf water potential (Ψ_{pd}), (b) percentage loss of hydraulic conductivity (PLC), (c) xylem water potential inducing 50% PLC (Ψ_{50}) and (d) safety margin calculated as the difference between Ψ_{50} and minimum seasonal xylem water potential (Ψ_{xyl}), as measured in *Quercus ilex* trees growing at four experimental sites (see Fig. 1). Mean values are reported (\pm SD). The regression lines together with r^2 and P values are also reported.

higher Ψ_{50} values at extensively paved sites than at more natural ones (Figs 7, 9c). Similar trends have been previously reported for plants exposed to chronic or extreme water stress, and have been interpreted as evidence of a ‘cavitation fatigue’ phenomenon (Hacke *et al.*, 2001) that would progressively increase vulnerability to cavitation, thus leading to hydraulic deterioration of the xylem system (Anderegg *et al.*, 2013; Nardini *et al.*, 2014). The lowest Ψ_{50} value recorded in the present study was -1.6 MPa (Fig. 7), and even in the case of VC elaboration by subtracting the PLC values recorded from fully rehydrated samples (*c.* 20%), Ψ_{50} would still remain *c.* -2.3 MPa. These values are in accordance with Ψ_{50} values reported in the literature for holm oak by Tognetti *et al.* (1998), but higher than more recent estimates for the species (e.g. -3.2 MPa; Pinto *et al.*, 2012). Although we cannot rule out that these differences are driven by genotypic plasticity, it has to be noted that Ψ_{50} variability across populations of woody plants has been suggested to be narrow in the few species tested (Wortemann *et al.*, 2011; Lamy *et al.*, 2014). On this basis, we suggest that the higher vulnerability to cavitation of urban holm oak trees subjected to the most intense and long-lasting water stress might be interpreted as evidence for hydraulic deterioration caused by cavitation fatigue. Indeed, the fact that some other studies focused on natural populations of holm oak found significantly more negative Ψ_{50} values than those reported here, would be in accordance with a ‘hydraulic degradation’ scenario for our study trees. Our data would also be in accordance with a study by Limousin *et al.* (2010) that reported increased vulnerability to cavitation in holm oak trees subjected to a 6-yr partial rainfall exclusion.

As a consequence of lower seasonal Ψ_{xyl} and intrinsically higher Ψ_{50} , the safety margin toward hydraulic failure (Choat *et al.*, 2012) was negative at those sites with large impervious surfaces, whereas it was slightly positive at the more natural sites (Fig. 9d). Although the physiological significance of the safety margin calculated over Ψ_{50} can be questioned, our findings do reveal that urban trees can easily surpass critical xylem water potential thresholds. This behavior might increase the risks of canopy dieback upon increased exposure to extreme drought and heatwaves (Helama *et al.*, 2012), and might indeed represent an important factor underlying the reported spatial patterns of tree mortality in urban areas, with higher die-off rates observed in areas with high soil compaction and/or impermeabilization (Nowak *et al.*, 2004). Our data suggest that appropriate design of urban green spaces, with special care given to limiting impermeable sealing of natural soil, might contribute to reduce the risk of tree decline, thus improving the effectiveness of mitigation strategies based on street tree planting.

Despite significantly different PLC levels recorded in August, native LSC was not different across sites, whereas LSC measured after embolism removal was higher in the most water-stressed trees (S_{90}), in accordance with similar data reported for holm oak by Limousin *et al.* (2010). This pattern was not due to compensatory changes in xylem anatomy, as vessel dimension and densities were not significantly different across sites. Maintenance of invariant LSC at increasing PLC was apparently achieved by reducing the leaf surface area supplied by stem xylem. Previous studies have also suggested that morphological adjustment is a key mechanism adopted by plants to match invariant or increased

transpiration demand with reduced soil water availability (Limousin *et al.*, 2010; Vaz *et al.*, 2012), and urban trees make no exception in this sense. From a practical point of view, adjustment of leaf area with increasing PLC might imply a reduction of canopy leaf surface area and, hence, a decrease in the shading provided by urban trees, which is key to the cooling effect of urban vegetation (Armson *et al.*, 2012). Hence, even though morphological adjustment through changes in biomass allocation might help urban trees to cope with drought, the urban ecological value of trees growing in the most stressful sites is likely to be reduced via hydraulic effects related to vulnerability to cavitation.

In conclusion, our data revealed new correlations between soil conditions, plant water status and hydraulic vulnerability of trees growing in urban areas. Growth conditions, with special reference to the amount of impermeable surface surrounding trees, influenced site-specific water status and drought vulnerability. However, data must be interpreted with caution, as several other confounding variables that might affect the physiology of urban trees were not explicitly investigated in this study. The study provides practical information for tree management in urban sites, but also suggests that key plant processes and responses such as drought-induced xylem cavitation, hydraulic deterioration and hydraulic adjustment are fundamental for a full understanding of responses of urban trees to environmental stresses. The study of urban tree hydraulics holds important promise in developing better adaptation strategies addressed at improving the sustainability of urban areas under ongoing climate changes.

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