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Stem photosynthesis contributes to non-structural carbohydrate pool and modulates xylem vulnerability to embolism in *Fraxinus ornus* L.

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ABSTRACT

Stem photosynthesis can significantly contribute to the carbon budget of woody plants, providing an extra carbon gain that might be crucial under drought stress causing leaf photosynthesis impairment and/or a reduced phloem transport.

Stems of *Fraxinus ornus* L. saplings were covered with aluminum foil to test the impact of inhibition of stem photosynthesis on plant vulnerability to drought. Plants were water-stressed to target xylem water potential of −3.5 MPa and were then re-irrigated to field capacity to quantify their recovery capacity. Vulnerability to xylem embolism was assessed in light-exposed and stem-shaded saplings with both the hydraulic method and *in vivo* with X-ray phase contrast micro-computed tomography. We also measured non-structural carbohydrate (NSC) concentration and osmotic potential in bark and wood, separately.

Stem shading increased xylem vulnerability to embolism formation under drought but did not influence the recovery phase. This difference was coupled with modification of the NSC pool and impaired osmoregulation, in particular in the wood of stem-shaded saplings compared to control ones.

Our results indicate stem photosynthesis as an important source of local NSCs, directly or indirectly involved in osmoregulation processes, which could be crucial to enhance the hydraulic resistance to embolism formation and to endure drought.

1. Introduction

Ongoing climate change is producing an increase in the frequency/severity of anomalous drought events (IPCC, 2022), leading to higher rates of tree dieback and mortality (Allen et al., 2010; Hember et al., 2017; Neumann et al., 2017). Drought-induced tree decline is mainly related to hydraulic failure and/or carbon starvation (McDowell et al., 2022). Since xylem and phloem functioning are strongly inter-related (Hölttä et al., 2009; Hubeau and Steppe, 2015), plant hydraulic and carbon dynamics are intimately linked (Adams et al., 2009; McDowell, 2011; Adams et al., 2017), so that these two critical events are likely to co-occur to some extent under drought (McDowell et al., 2011; McDowell et al., 2015; Lloret et al., 2018). Hence, when considering the effects of drought stress on plant survival, it is fundamental to consider both processes (Nardini et al., 2011; Sevanto et al., 2014; Adams et al., 2017). A recent review highlighted the relationships between plant hydraulics and non-structural carbohydrate (NSC) dynamics (Tomasella et al., 2019b), as mediated by water and solutes exchanges between xylem conduits and parenchyma cells where NSCs are stored.

Leaves are the primary photosynthetic organs for most woody species, and are generally assumed to be the major source of carbon assimilation. Nonetheless, a wide range of woody plants can carry out photosynthesis even at stem level (Pfanz and Aschan, 2001), which can be considered as an extra source of carbon assimilation that can help plants to face drought events. Generally, the spatial distribution of chloroplasts within stems strongly depends on the interspecific characteristics in axial and radial light transmission through stem tissues (Sun et al., 2003, 2005; Schmitz et al., 2012), which generally depends on bark features (Aschan et al., 2001; Cernusak and Cheesman, 2015). The functional advantage of stem photosynthesis lies in the fact that it can be

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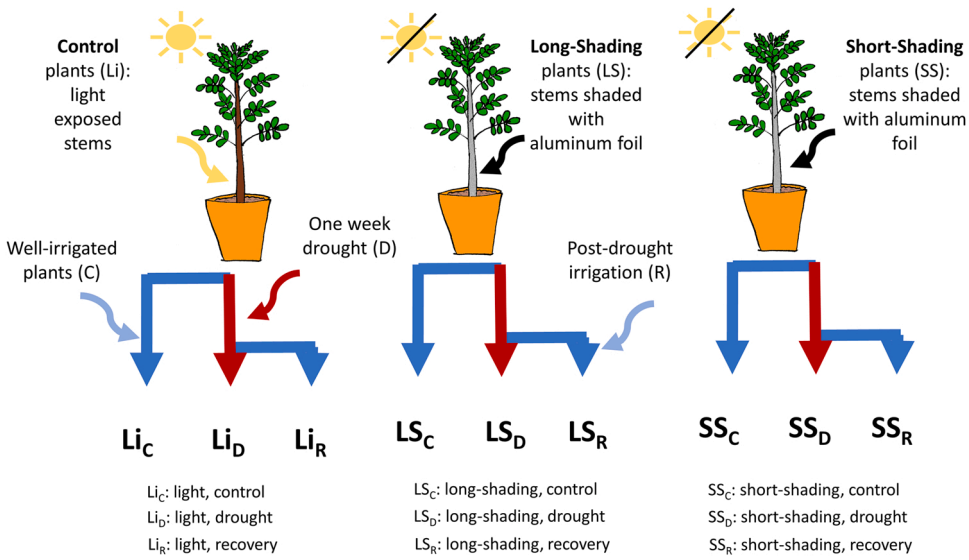

Fig. 1. Schematic representation of the experimental design. *Fraxinus ornus* plants with stems light-exposed (Li) and shaded (LS, SS) are shown with brown and grey stems respectively. Blu and red arrows indicate irrigation and drought treatments. The drought-recovery experiment aimed to compare control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D), and recovery (Li_R, LS_R, SS_R) plants.

sustained either from the external CO_2 uptake (at bark level) and/or from the re-fixation of CO_2 released by respiration (Pfanz et al., 2002; Teskey et al., 2008; Ávila et al., 2014). Indeed, stem photosynthesis has been reported to substantially contribute to wood production, thus playing a key role in stem growth (Saveyn et al., 2010; Cernusak and Hutley, 2011; Bloemen et al., 2013; Steppe et al., 2015), and bud development (Saveyn et al., 2010). As an example, stem photosynthesis was reported to contribute to stem growth by 25–56 % in three evergreen woody species (Saveyn et al., 2010), by about 10 % in mature *Eucalyptus* species (Cernusak and Hutley, 2011), and by 30 % in *Populus tremuloides* (Bloemen et al., 2016).

The peculiar capacity of stems to rely on endogenous CO_2 (Pfanz et al., 2002; Aschan and Pfanz, 2003; Wittmann and Pfanz, 2008) might represent an important advantage when leaf photosynthesis is impaired by stomatal closure and phloem transport is limited (Vandegehuchte et al., 2015). The photosynthetic activity of stems might also minimize the impact of absence of leaves due to seasonality (e.g. deciduous species), and might become particularly important under stress conditions (e.g. drought stress) (Teskey et al., 2008; Eyles et al., 2009). Indeed, the first observations of photosynthetic stems were for desert plants (Gibson, 1983; Nilsen, 1995) and recent studies have suggested a coordination between stem photosynthesis and hydraulics in these species (Ávila-Lovera et al., 2017).

The first clear evidence of an involvement of stem photosynthesis in hydraulic functioning was provided by Schmitz et al. (2012), who highlighted a reduction in stem hydraulic conductivity in detached branches of different mangrove species after branches were covered to block light penetration. This led to the hypothesis that the inhibition of stem photosynthesis induced a depletion of carbohydrate reserves with consequent failure of embolism repair processes (Nardini et al., 2018). Moreover, inhibition of stem photosynthesis can induce stems to shrink more drastically in response to severe drought stress (Bloemen et al., 2016). Hence, sugars produced at stem level might potentially be involved in maintaining the turgor of wood parenchyma cells, which are supposed to assist xylem hydraulic functioning (Secchi et al., 2021; Tomasella et al., 2021; Kawai et al., 2022). Similarly, light-excluded branches of *Populus nigra* displayed a steeper increase in embolism formation for any given water potential, compared to control stems (De Baerdemaeker et al., 2017). A recent study pointed out that stem shading had a different impact on an evergreen woody species (*Laurus nobilis*) compared to a deciduous one (*Populus alba*) (Trifilò et al., 2021). Specifically, the inhibition of stem photosynthesis affected the growth of *Laurus nobilis* but not of *Populus alba*. Conversely, stem shading affected the ability to recover from xylem embolism in *P. alba*, but not in *L. nobilis*.

All the above studies suggest that stem photosynthesis might have an impact on hydraulic functioning under drought, or on recovery capacity after drought release. An important unresolved issue concerns the contribution of stem photosynthesis to the NSC pool in the wood, and how this interacts with hydraulic responses during and after drought. In this study, we report a set of experiments aiming to test the impact of long- and short-term stem shading on hydraulic responses to drought of *Fraxinus ornus* L. This species was selected because it is known to have functional chloroplasts in bark and wood (Natale et al., 2023), and because it is a drought-tolerant tree facing prolonged summer periods with partial or full stomatal closure (Nardini et al., 2003; Tomasella et al., 2019a).

2. Material and methods

2.1. Experimental design and plant material

Three-year-old potted saplings of *F. ornus* (n = 83) were provided by a public nursery (Vivai Pascul, Regional Forestry Service, Tarcento, Italy). Plants were transplanted on February 2020 in 3.5 L pots filled with a light mineral substrate designed for green roof installations (Savi et al., 2013), and randomly allocated in a greenhouse of the botanical garden of the University of Trieste (Italy). The plant's height (h) and the diameter at the stem base (ø) were measured both at the beginning and end of the experiment with a meter and a caliper, respectively.

Plants were regularly irrigated to field capacity until the start of experimental drought treatments, and their position in the greenhouse was randomly shifted weekly, to assure exposure to uniform light conditions. A slow-release fertilizer (Flortis, universal fertilizer, Orvital, Milano, Italy) was added to each pot in April (4 g) and May (3 g) to prevent nutritional deficiency. Saplings were divided in three groups (ca. 30 plants each) (Fig. 1): i) plants maintained under greenhouse light conditions for the whole experiment (Li, Light plants); ii) plants with stems covered with aluminum foil for 30 days before starting the drought experiment (LS, Long-shading plants); iii) plants with stems covered with aluminum foil at the beginning of the drought experiment (SS, Short-shading plants). The shaded stems were loosely wrapped in

aluminum foil to prevent stem photosynthesis while allowing gas exchange with the atmosphere, as also reported in De Baerdemaeker et al. (2017). By covering the whole stem to inhibit stem photosynthesis, we aimed at favoring the depletion of local non-structural carbohydrate (NSC) reserves. For each group, plants were further divided into three treatments: i) well-watered (C, control), ii) drought-stress (D), and iii) recovery (R). The drought stress was induced by suspending irrigation for 5–10 days, until the xylem water potential (Ψ_{xyl}) dropped to about −3.5 MPa, i.e. a value known to induce 50 % loss of xylem hydraulic conductance due to embolism in this species (Petit et al., 2016). Plants for the recovery experiment were re-irrigated to field capacity and harvested 24 h later to assess eventual occurrence of hydraulic recovery. C plants (Li_C, LS_C, SS_C) were harvested and measured within the same period as D and R plants. For each group, five to ten plants were harvested for pigments quantification as well as hydraulic and NSC measurements between 12:00 and 14:30 h, to avoid diurnal fluctuation effects on carbohydrate content (Tixier et al., 2018).

2.2. Leaf water relations and stem osmotic potential

To assess eventual effects of water and/or light treatments on plant water status, we measured midday stem xylem water potential (Ψ_{xyl}), leaf conductance to water vapor (g_L), and stem osmotic potential. In particular, g_L (mmol m^{-2} s^{-1}) was measured with a steady-state porometer (SC-1, Decagon Devices Inc., Pullman, USA) between 11:00 h and 14:00 h on 2 mature and healthy leaves per sapling. Measurements were coupled to quantification of photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) with a Quantum photo-radiometer (HD 9021, Delta OHM S.r.l., Padova, Italy) at the selected leaf surface.

To assess Ψ_{xyl}, 1–2 mature leaves per sapling were bagged in cling film and covered with aluminum foil in the early morning. Leaves were then collected between 11:00 h and 14:00 h and water potential was measured using a pressure chamber (mod. 1505D, PMS Instrument co., Albany, OR, USA).

Additionally, stem osmotic potential at full turgor was estimated on 5–10 saplings for each group/treatment. Bark and wood were manually separated incising the bark with a razor blade and peeling it from the wood, re-hydrated for 20 min by immersion in bi-distilled water, wrapped in parafilm and stored at −20 °C until measurements. Samples were thawed for c. 10 min, chopped quickly and wrapped in cling film, and placed in liquid nitrogen for 60 s. Half of the sample was put immediately in a dewpoint hygrometer to measure the osmotic water potential (WP4, Decagon Devices Inc., Pullman, USA), and the other half was stored in 1 mL Eppendorf vials filled with bi-distilled water, and shaken for 30 s before measuring the electrical conductivity (C, μS cm^{-1}) of the solution with a conductivity meter (LAQUAtwin-EC-11, Horiba Ltd., Kyoto, Japan). Data collected during the greenhouse experiment were combined with those obtained during the micro-CT experiment (see below).

2.3. Pigment analysis

To investigate the possible effects of stem shading and water treatments on pigment concentration, we quantified chlorophyll *a*, chlorophyll *b* and carotenoids content in both bark and wood upon extraction of fresh material in 80 % acetone solution. Bark and wood samples (5–10 saplings for each group/treatment) were cut into small pieces to facilitate the extraction. About 50 mg of bark samples were placed in a 2 mL Eppendorf filled up with 1.5 mL of acetone solution. About 300 mg of wood were placed in 1.5 mL of acetone solution. Absorption spectra were recorded using an UV-Vis spectrophotometer (model 7315; Jenway, Worcestershire, UK) between 250 and 750 nm. The final pigment concentration was assessed using Wellburn equations (Wellburn, 1994).

2.4. Hydraulic measurements

In order to quantify eventual impacts of stem shading on xylem vulnerability to embolism or on post-drought hydraulic recovery, we measured the percentage loss of hydraulic conductivity (PLC) in one-year-old stem segments in Li, LS, and SS saplings (5–10 saplings per group/treatment). PLC was measured in well-watered saplings, as well as in plants exposed to D and R treatments.

To minimize any possible excision artifact in PLC quantification, we followed the protocol by Torres-Ruiz et al. (2015). We optimized the method by making the first cut underwater. Whole pots wrapped in a plastic bag were immersed in a bucket filled with tap water, and the stem was cut at the base under water. Then, the stem was transferred in clean tap water and a second cut was made at a minimum distance of 0.2 times the maximum vessel length (MVL). The residual xylem tension was released by immersing the cut end in water for 20 min. MVL was estimated with the "air injection method" described by Wang et al. (2014), and averaged 23 ± 9 cm in our saplings. Then, the xylem segment to be used for hydraulic measurements was excised under water at the minimum distance of 1.5 MVL from the initial cut, to remove any artificial embolism generated during previous cuts. Hydraulic conductance was measured with a hydraulic apparatus (Tomasella et al., 2019a), using filtered (0.45 μm) and degassed mineral water added with 10 mM KCl as perfusion solution (Nardini et al., 2007). Stem hydraulic conductance was measured gravimetrically under a water head of 4 kPa before (initial hydraulic conductance, K_i) and after (maximum hydraulic conductance, K_{max}) flushing the sample at high pressure (0.15 MPa) for 3 min to remove embolism. Maximum stem specific hydraulic conductivity (kg m^{-1} s^{-1} MPa^{-1}) was calculated as:

$$K_s = K_{max} \text{ x } l/A_x$$

where l is sample length and A_x is the xylem transverse area, as calculated on the basis of the radius of the stem cross section area occupied by xylem, measured using a caliper.

Finally, PLC was calculated as:

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

2.5. Micro-CT imaging

In order to validate results obtained with destructive hydraulic techniques, we performed phase contrast micro-CT scans on intact saplings (n = 26) at the SYRMEP beamline of the Elettra Synchrotron light source (Trieste, Italy), on 17–21 July 2020. One month before Synchrotron access, the whole stems of 12 plants were shaded with aluminum foil (LS, long-shaded plants), whereas other 12 plants were kept under ambient light conditions (Li, light plants). Eight plants per group were subjected to drought stress by withholding irrigation until Ψ_{xyl} reached the critical threshold (see above). Stems were quickly fixed to the sample holder and the plant was wrapped in cling film to avoid water loss. For image acquisition, the average X-ray energy was 22 keV and pixel size was set at 2 μm to clearly visualize xylem conduits. The exposure time was 200 ms (ms) per image, except for some taller saplings where this value was set at 100 ms to reduce plant movement during rotation. Scans were performed over 180°, with 1800 projections per sample. The slice reconstructions were carried out using SYRMEP Tomo Project (STP) software (Brun et al., 2015). A phase retrieval pre-processing algorithm (Paganin et al., 2002) was applied prior to the conventional filtered back-projection algorithm to increase the image contrast. Reconstructed images were processed using ImageJ. Due to the larger diameter of the stems compared to the field of view (ca. 4 mm × 4 mm), analyses were conducted on about one-quarter of the stem section, excluding the immature xylem next to the vascular cambium. The embolized sapwood area (A_{embol}) was calculated by dividing

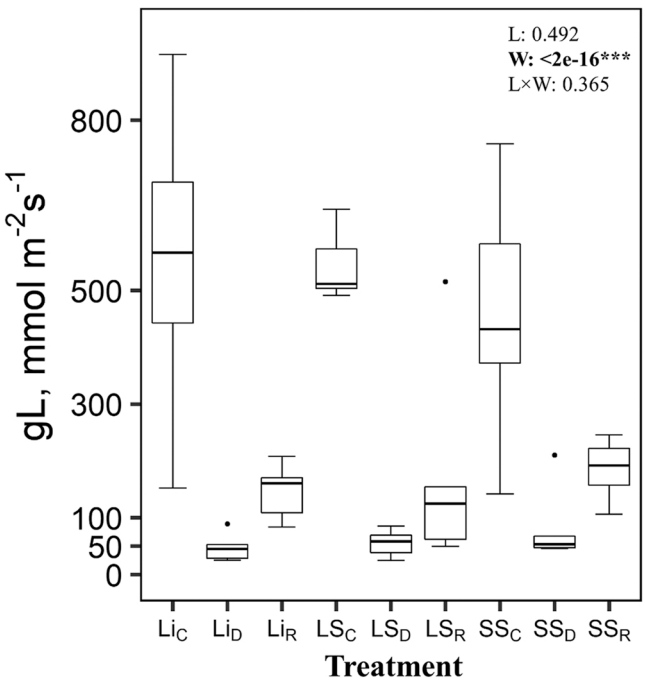

Fig. 2. Median values, 25th and 75th percentiles of leaf conductance to water vapour (g_L) measured in light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings at midday. Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

the total embolized pixel area by the analyzed mature sapwood area, and expressed as percentage (Secchi et al., 2021).

2.6. Stem non-structural carbohydrate content

In order to investigate the effects of the experimental treatments on carbohydrate dynamics, NSC analysis was performed on 4 cm long stem segments, sampled next to those used for hydraulic measurements immediately after cutting (5–10 saplings for each treatment). Bark and wood were separated, microwaved for 3 min at 700 W in order to stop enzymatic activity, oven-dried at 70 °C for 24 h, then grinded with a mixer mill (MM400; Retsch GmbH, Haan, Germany), and stored at −20 °C until NSC extraction. Aliquots of 15 ± 2 mg of powder were processed following Tomasella et al. (2019a), with the following modifications: after extraction with 80 % ethanol, soluble carbohydrates were recovered in the alcoholic supernatant. Soluble NSCs were measured as mg g^{-1} DW of glucose by the Anthrone assay method (Yemm and Willis, 1954), comparing sugars content of samples with known amount of glucose. The pellet was incubated in 0.5 mL 2 mM HEPES-Tris pH 6.7 at 25 °C overnight to extract maltodextrins and other oligosaccharide fractions. The evaluation followed the same protocol of above. The pellet was processed according to Landhäusser et al. (2018) to measure starch. Hydrolysis was performed according to Landhäusser et al. (2018). The glucose resulting from the starch enzymatic digestion was quantified as NADPH using a spectrophotometer (Perkin Elmer, Boston, MA, USA), following Bergmeyer and Bernt (1974). Starch amount was expressed as mg of glucose equivalent per g of DW, by comparison with standard solutions of amylose that followed the same processing of samples.

2.7. Statistical analyses

Statistical analyses were carried out with R (R Core Team, 2022). Boxplot graph were prepared with the 'ggplot2' package. We used

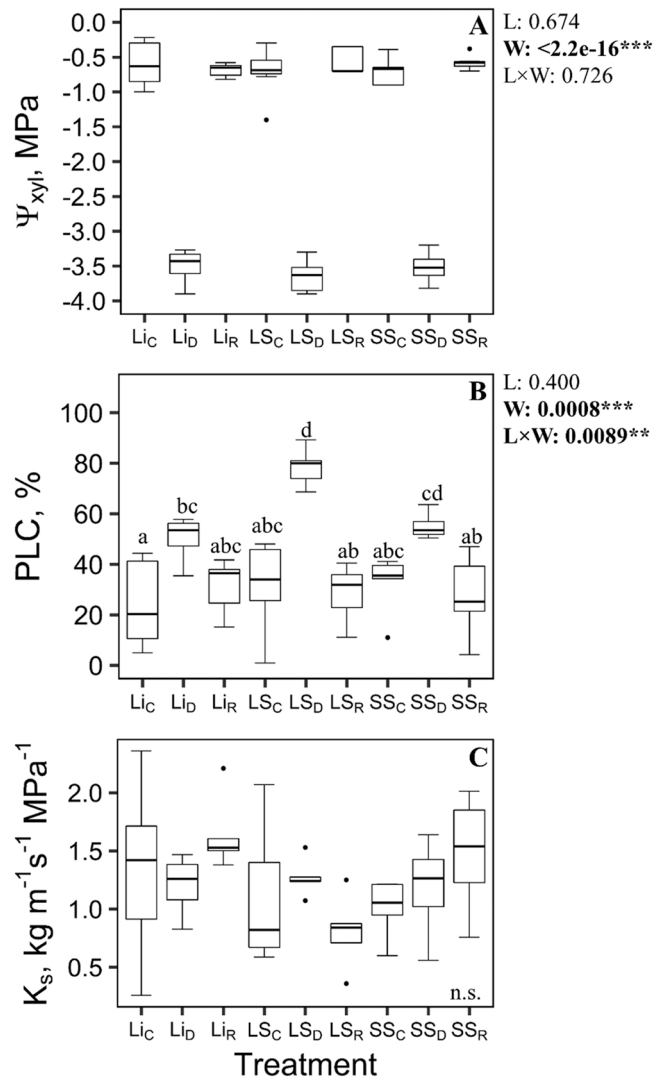

Fig. 3. Median values, 25th and 75th percentiles of A) xylem water potential (Ψ_{xyl}), B) percentage loss of hydraulic conductance (PLC) and C) maximum stem specific hydraulic conductivity (K_s) measured in light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings. Different letters indicate statistically significant differences among treatments ($p < 0.05$). n.s. = not statistically significant. Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

generalized least squares (GLS) analyses ('nmle' R package), setting each parameter measured (e.g. g_L, Ψ_{xyl}, π_0, etc) as the response variable and the light treatment (L) and the water treatment (W) as the first and second explanatory variables, respectively. When homogeneity of variance assumption was violated we used the "varPower()" variance structure (Pinheiro et al., 2016). When the interaction was statistically significant ($p < 0.05$), differences between groups were tested and p-values were adjusted using the Holm correction through the emmeans function in 'emmeans' R package.

3. Results

Plant height (h) and diameter at the stem base (ø) measured both at the start and end of the experiment are reported in Table S1. Plant size was similar in all experimental groups, and did not change significantly along the duration of the experiment.

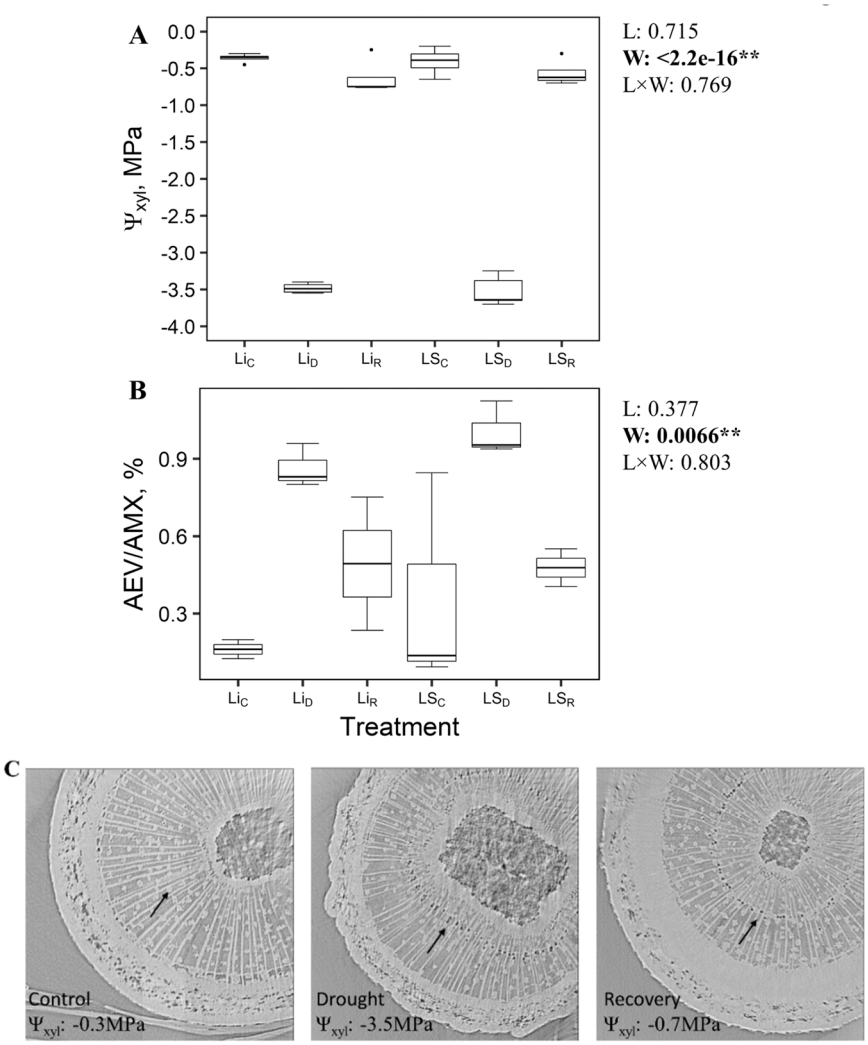

Fig. 4. Median values, 25th and 75th percentiles of A) xylem water potential (Ψ_{xyl}), B) percentage of total area of embolized vessels (AEV) over total area of mature xylem (AMX) in response to changes in xylem pressure during drought and recovery treatments. Data were measured in light (Li) and long-shaded (LS) control (Li_C, LS_C), drought (Li_D, LS_D) and recovery (Li_R, LS_R) *Fraxinus ornus* saplings. Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$. C) In vivo visualization by X-ray microtomography (micro-CT) in stems of intact *Fraxinus ornus* saplings. Reconstructed cross-sections showing embolized (air-filled vessels, dark circles) and functional conduits (water-filled, light gray circles) in well-watered, stressed and recovered plants, respectively.

3.1. Stem shading's effects on pigments concentration

As reported in Fig. S1, we did not observe any significant effect of the interaction of light and water treatments on chlorophyll and carotenoid concentration in the bark. A significant effect of light treatment in Chl*a* and Chl*b* concentration of wood samples was observed. In particular, compared to LS_C plants, SS_C ones showed a slightly higher amount of total chlorophyll concentration (Chl *a*+*b*) (Fig. S2, Appendix 1). Nonetheless, it is worth noting that Chl*a*, Chl*b* and Car concentration tended to be lower in bark samples of both shaded treatments, with a marked decrease for LS ones (Fig. S1).

The investigated interaction (Light × Water, Fig. S2) was significant for Chl *a/b* in wood and for carotenoids/total chlorophyll (*a*+*b*) ratio (Car/Chl tot) in bark. LS_D plants had overall highest Chl *a/b* in wood, and SS_C plants had lowest Car/Chl tot.

3.2. Plant water relations

Midday values of g_L and Ψ_{xyl} during the experimental period were statistically similar in well irrigated light (Li) and shaded (LS, SS) samples (Figs. 2, 3A respectively). Drought induced a drop in Ψ_{xyl} that reached ca. −3.5 MPa in all light treatments, leading to marked stomatal closure. Upon recovery, plant water status returned to the values detected for controls, but g_L remained lower than values measured before drought.

Values of PLC for well irrigated plants averaged 20–30 % in all light treatments. Despite similar drops of Ψ_{xyl} (Fig. 3A) under drought, hydraulic measurements showed that PLC was markedly higher in LS_D plants compared to Li_D ones (50.0 ± 10.1 and 78.6 ± 7.8 %, respectively). A non-significant tendency toward higher PLC values was detected also in SS_D plants compared to Li_D. Upon re-irrigation, PLC returned to control values within 24 h, and stem shading apparently did not affect the ability to recover xylem hydraulic function (Fig. 3B, Appendix 1). No differences were detected in terms of maximum stem hydraulic conductivity across different groups/treatments (Fig. 3C).

Micro-CT analysis of xylem in intact plants allowed to identify water-filled (functional) and gas-filled (non-functional) vessels (Fig. 4C) in saplings exposed to different light and water treatments. Changes in AEV/AMX are shown in Fig. 4B. Changes in plant water status in C, D and R plants were similar to those detected in the main experiment (Fig. 4A). We observed that the number of embolized vessels increased in drought-treated plants compared to controls, but these apparently returned to the functional status upon re-irrigation. Consistently with results obtained in hydraulic measurements, LS_D plants had higher embolism levels compared to Li_D ones, although the low number of samples masked the statistical significance in this set of plants.

Electrical conductivity was similar in all groups/treatments, both in bark and wood (Fig. 5). Osmotic potential (Fig. 6) was also similar across experimental groups at bark level. However, a different pattern of osmoregulation was observed at wood level in light vs shaded plants. In fact, under drought stress only plants with light exposed stems (Li) adjusted the wood osmotic potential at full turgor (π_0) to more negative

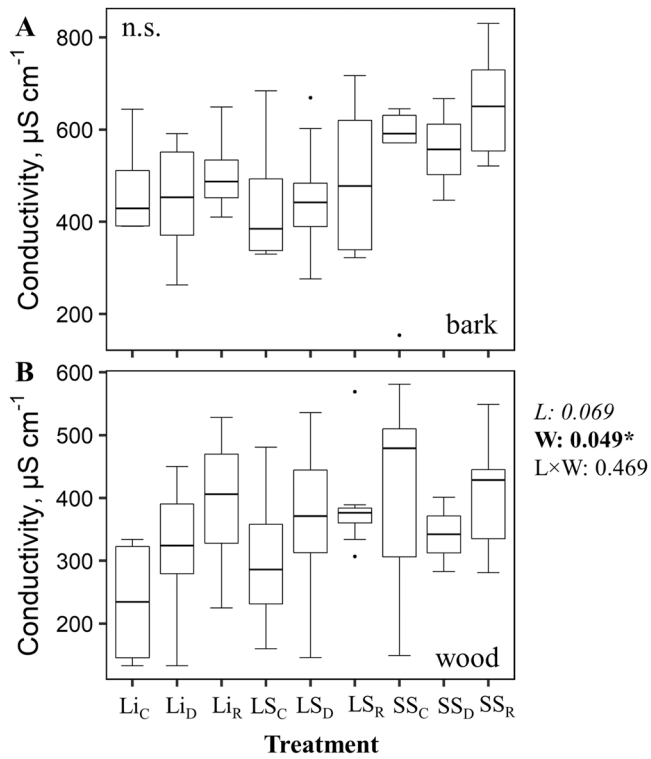

Fig. 5. Median values, 25th and 75th percentiles of A) bark and B) wood conductivity, measured in light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings. n.s. = not statistically significant. Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

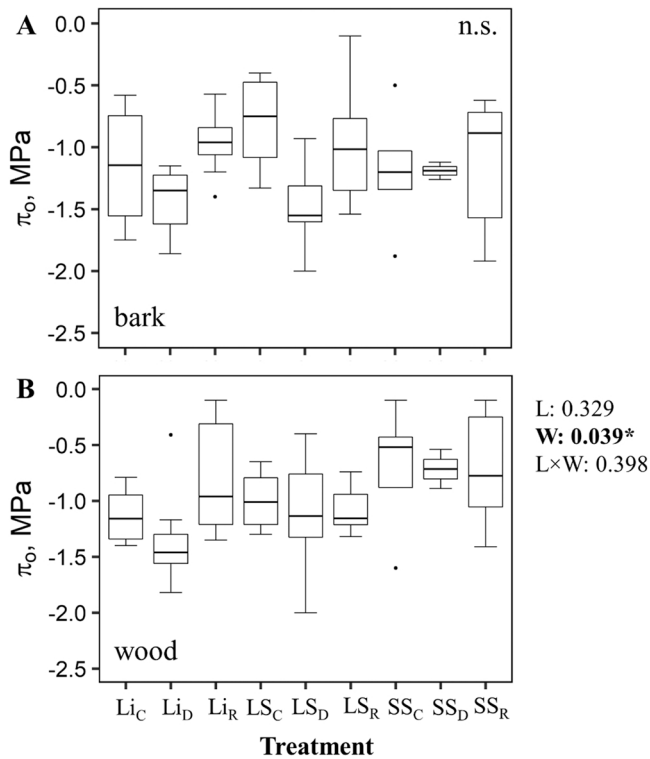

Fig. 6. Median values, 25th and 75th percentiles of A) bark and B) wood osmotic potential at full turgor (π_0), measured in light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings. n.s. = not statistically significant. Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

values than control plants (Fig. 6B). Interestingly, SS plants had the highest (less negative) osmotic potential, although the high variability of data masked any possible statistical significance.

3.3. Stem non-structural carbohydrates content

In general, Li plants showed different trends of NSC dynamics/content compared to LS and SS plants, especially under drought and in particular at wood level (Figs. 7, 8). Starch contents were tendentially lower in the wood of LS_C and SS_C plants compared to Li_C ones (Fig. 7D). Moreover, stem shading significantly affected the ability of LS and SS sample to mobilize the pool of NSCs (starch + soluble sugars) in both bark and wood under drought stress, when compared to Li samples (Fig. 8). In fact, drought induced a decrease of total NSC content both in the bark and wood only in Li plants, mainly due to the marked starch depletion (Fig. 7B, D), while LS and SS plants showed NSC levels under well-watered conditions that remained unchanged under drought, while showing a decrease under recovery. The described diverse dynamics of stem NSCs in plants exposed to shading and drought is confirmed by the significant interaction (LxW) observed in the pool of NSCs (Fig. 8).

4. Discussion

Our data provide new insights on the roles played by stem photosynthesis in plant responses to drought stress. We found that plants exposed to a long-term stem shading became more vulnerable to xylem embolism during drought compared to control plants, but no effect of stem shading was recorded during the recovery phase. Changes in xylem hydraulic vulnerability were coupled with modifications of the NSC pool and impaired osmoregulation processes, suggesting a possible role of parenchymatic cell turgor in xylem hydraulic functioning.

Stem shading had no impact on gas exchanges rates in well-irrigated plants, and under drought the magnitude of xylem water potential drop and g_L reduction was similar in all light treatments. Upon re-irrigation, plant water status returned promptly to control values but g_L remained low, at least 24 h after water re-supply. This might suggest some residual hydraulic limitation to gas exchange, or the presence of a chemical signal super-imposed on the hydraulic one, limiting g_L recovery. We can probably rule out the first hypothesis, as hydraulic measurements revealed that stem hydraulic functioning promptly returned to pre-drought levels upon re-watering (see below). Hence, any residual hydraulic limitation could have been located at the root or stem level. It is also possible that chemical signals, e.g. ABA or malate, which can be found in *Fraxinus* xylem sap and have been suggested to regulate stomata closure (Patonnier et al., 1999), accumulated in leaves under drought and were not promptly removed or de-activated upon re-irrigation. Similar observations have been previously reported for different woody species (Loewenstein and Pallardy, 2002; Martorell Lliteras et al., 2014; Tombesi et al., 2015; Hasan et al., 2021), suggesting the presence of a mechanism delaying stomatal aperture in some species, even after apparent recovery of hydraulic functioning and plant water status. This mechanism might be important to prevent any water potential drop before embolism repair processes have been completed, considering that hydraulic recovery is energetically favored only when xylem water potential rises to near-zero values (Nardini et al., 2018).

Despite similar trajectories in terms of gas exchange and water status, saplings subjected to different light treatments showed different patterns of embolism accumulation and loss of hydraulic conductivity when exposed to drought conditions. In particular, PLC was about 30 % in all groups when under full irrigation, and slightly increased to about 50 % in Li_D plants. On the contrary, PLC markedly increased in LS_D plants (up to 80 %) and SS_D ones (about 60 %). These findings suggest

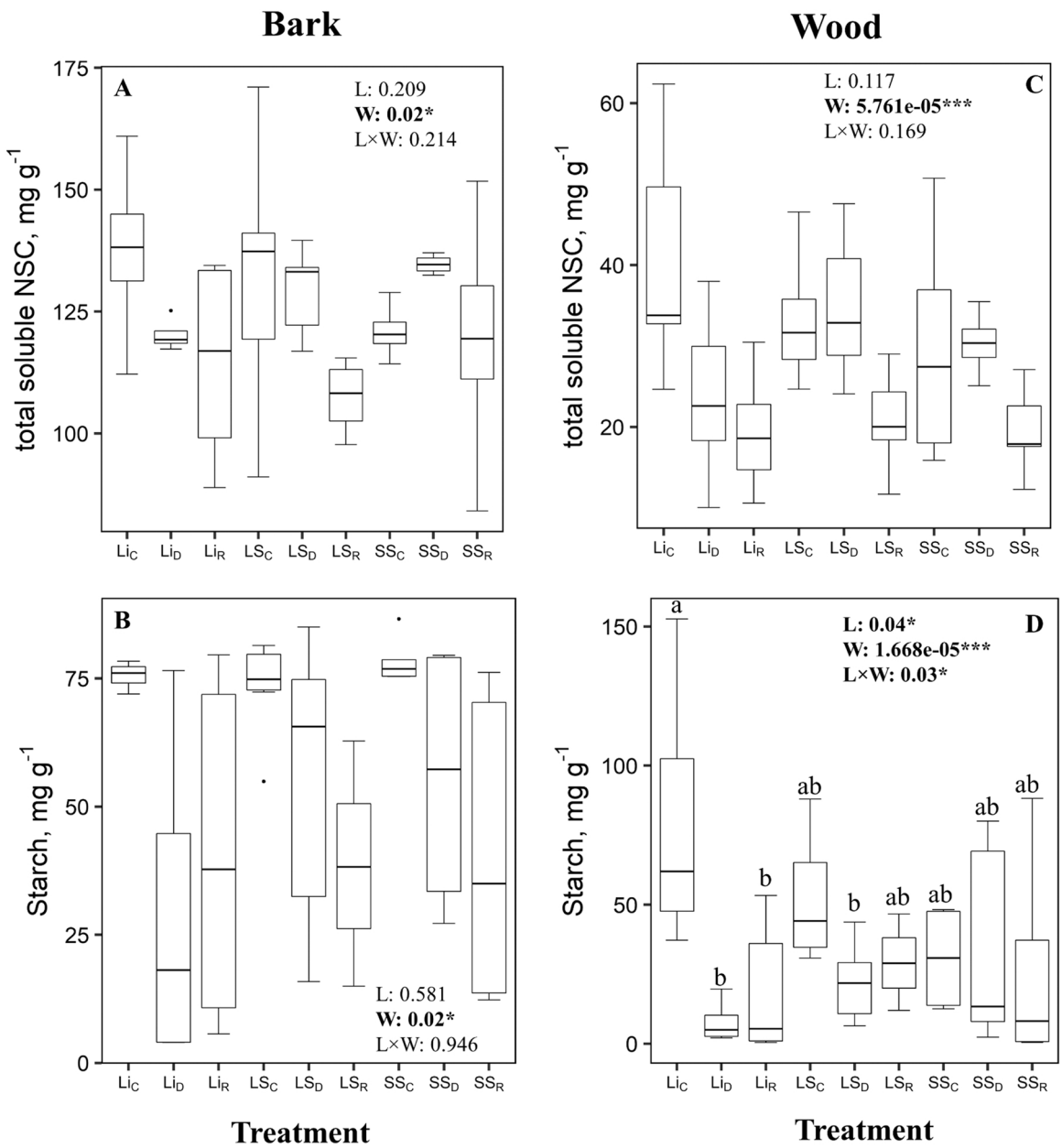

Fig. 7. Median values, 25th and 75th percentiles of A, C) stem total soluble non-structural carbohydrates (NSC) and B, D) starch measured in bark (A, B) and wood (C, D) of light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings. Different letters indicate statistically significant differences among treatments ($p < 0.05$). Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

that stem shading increased the vulnerability to xylem embolism, making plants more prone to the risk of hydraulic failure under drought. Our data are consistent with previous findings (Schmitz et al., 2012; Bloemen et al., 2016; Chen et al., 2021) and confirm that stem photosynthesis is an important modulator of plant hydraulic performance.

Changes in xylem anatomy induced by different light conditions can influence plant hydraulics (Plavcova et al., 2011). Although we did not measure anatomical traits in our study plants, measurements of plant size at the start and the end of the experiment did not show any active growth during this period. Hence, it is unlikely that differences between groups/treatments derived from the production of new xylem conduits with different features. This is also confirmed by the fact that maximum stem hydraulic conductivity was similar in all experimental groups and treatments (Fig. 3C). However, we cannot rule out the possibility that shading induced modifications in the ultrastructure of pit membranes, as reported in some species (Plavcova et al., 2011) but not in others (Tomasella et al., 2021), with potentially important impacts on conduits' vulnerability to air-seeding (Thonglim et al., 2021). However, changes in pit membrane's structure should also translate in changes of xylem hydraulic efficiency, which was not the case in our study plants.

The observed increase in vulnerability to xylem embolism induced by stem shading (Fig. 3) is intriguing, but in line with previous observations (De Baerdemaeker et al., 2017; Tomasella et al., 2021). Previous studies have suggested that a reduction in NSC availability induced by inhibition of stem photosynthesis would be at the basis of this phenomenon, and our data indeed confirm that NSC pool was reduced in the wood of shaded plants compared to controls. Furthermore, control plants showed a significant depletion of carbohydrates under drought, while the drop was relatively minor in the NSC-limited shaded stems (Fig. 7). The mechanistic link between NSC availability and xylem

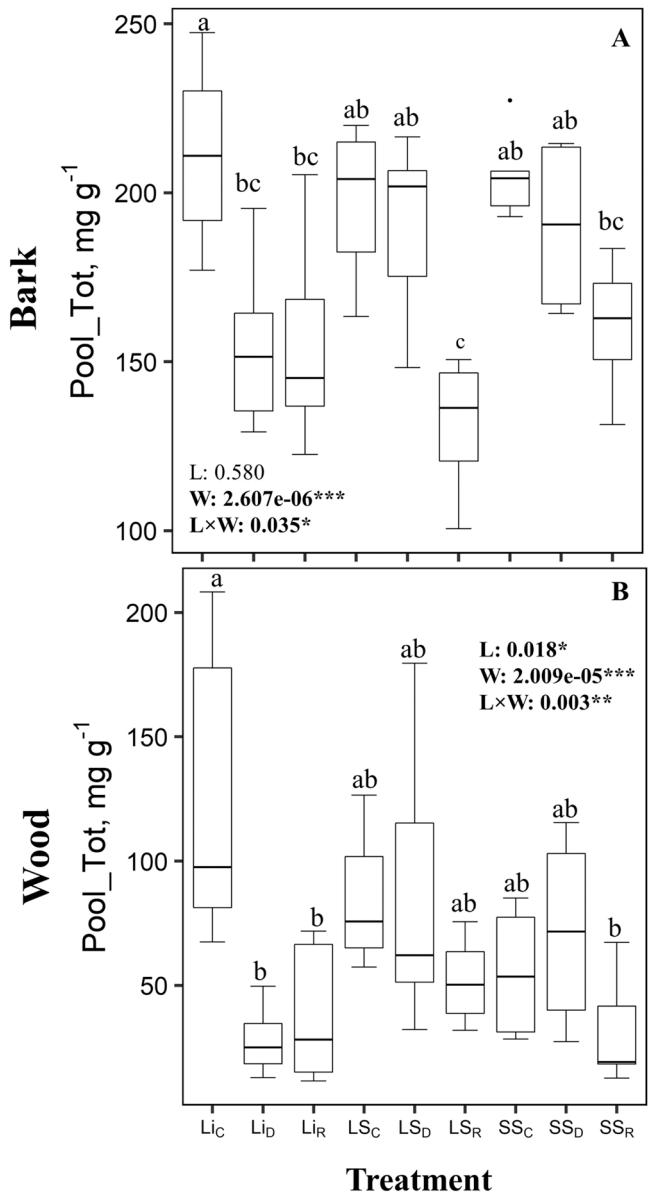

Fig. 8. Median values, 25th and 75th percentiles of stem total non-structural carbohydrates (Pool Tot) measured in A) bark and B) wood of light (Li), long-shaded (LS) and short-shaded (SS) control (Li_C, LS_C, SS_C), drought (Li_D, LS_D, SS_D) and recovery (Li_R, LS_R, SS_R) *Fraxinus ornus* saplings. Different letters indicate statistically significant differences among treatments ($p < 0.05$). Explanatory variables (light treatment, L, and water treatment, W) and associated p-values are reported. *= $p < 0.05$; ** = $p < 0.01$; ***= $p < 0.001$.

hydraulics remains elusive, although different hypotheses have been proposed. NSCs might be required to modulate the surface tension of xylem sap, which can indeed affect xylem vulnerability (Losso et al., 2017). Moreover, NSCs might represent energy sources for the synthesis of lipids and proteins that have been suggested to act as surfactants and stabilizers of gas nanobubbles in the xylem sap (Schenk et al., 2017). A third hypothesis was advanced by Tomasella et al. (2021), suggesting that NSCs might be crucial for maintaining cell turgor and continuity between wood parenchyma cells and both phloem cells and xylem conduits under drought (De Roo et al., 2020). Indeed, vessel-associated cells (VACs) are known to exchange water and solutes with the xylem conduits (Morris et al., 2018). Under drought, it is likely that the water potential of VAC equilibrates with the negative pressure inside the conduits, possibly leading to cell turgor loss and shrinkage (Oparka, 1994). In *F. ornus* the turgor loss point of leaf cells shifts on a seasonal basis from −2.5 MPa in spring to −3.2 MPa by the end of summer drought (Nardini et al., 2003). This last value would be close the Ψ_{xyl} value of −3.5 MPa targeted in our drought experiment. Hence, it is possible that wood parenchyma of LS_D (and SS_D) plants experienced the risk of turgor loss and cell shrinkage due to lack of NSCs and limited osmoregulation capacity, possibly increasing the risk of air seeding at the interface between VAC and xylem conduits. In fact, we observed a decrease in stem osmotic potential only in Li_D plants (Fig. 6), but not in LS_D or SS_D. Interestingly, changes in osmotic potential were not accompanied by significant changes in electrical conductivity, suggesting that osmoregulation was achieved at least partly via accumulation of sugars or other non-charged compounds, that were less available (and not mobilized under drought) in both LS and SS plants.

Previous studies have suggested that the inhibition of stem photosynthesis can lead to an impairment of post-drought hydraulic recovery processes (Schmitz et al., 2012; Bloemen et al., 2016; Liu et al., 2019). Embolism repair is an energy-dependent process still not fully elucidated. On the basis of available observations, the process is likely based on an osmotic mechanism that involves release of sugars into refilling conduits (Savi et al., 2016; Tomasella et al., 2017; Secchi et al., 2021). Hence, a reduction in NSC pools as induced by inhibition of stem photosynthesis is expected to delay or prevent the recovery of hydraulic functions after re-irrigation (Trifilò et al., 2017). However, in our set of plants complete hydraulic recovery was observed in all light treatments. Changes in PLC under drought, as estimated on the basis of destructive hydraulic techniques, can arise because of artefacts caused by excision of stem samples while the xylem conduits are under substantial tension (Wheeler et al., 2013). On the other hand, relaxation procedures aimed at preventing such artefacts can induce artificial refilling of xylem conduits, more likely when xylem tension values are moderate (Trifilò et al., 2014). We can rule out the possibility of such artefacts in our dataset, because the same pattern of embolism build-up under drought and recovery after re-irrigation was observed in intact plants, based on micro-CT analysis. This let us speculate that hydraulic recovery was possible also in LS and SS plants, either because the residual amount of NSCs was still sufficient to sustain the process, or because xylem tension transiently increased to near-atmospheric values under low-transpiration conditions, favoring spontaneous dissolution of the gas phase in xylem conduits (Konrad et al., 2018). A transient generation of root pressure after re-irrigation might also explain the hydraulic recovery observed in this study (Knipfer et al., 2015; Schenk et al., 2021), although *F. ornus* is not among the species known to produce substantial root pressure (Schenk et al., 2021).

In conclusion, our results confirm that NSCs produced by stem photosynthesis are modulators of plant hydraulic performance under drought stress, favoring the long-term maintenance of xylem hydraulic function. We suggest that these effects are mediated by the direct or indirect role of NSCs in osmoregulatory processes, but the mechanistic link between turgor loss of wood parenchyma and embolism build-up still awaits to be elucidated. In any case, our data suggest that enhanced photosynthetic capacity at stem level might be a trait to be considered and tested for selection of woody crops and forest trees with improved tolerance to extreme drought events.

CRediT authorship contribution statement

SN, AN, MT, and FP designed the experiment; SN performed the experiment and measurements, with help from SG, MT, FP, EB, VC and AN; SN and MT performed hydraulic measurements; EB performed leaf conductance to water vapor measurements; SN, SG and VC performed NSC extraction, analysis and data interpretation; SN, MT, FP, GT and AN performed the CT scans at the Synchrotron; SN and FP analyzed the data; SN and AN wrote the manuscript, with contributions and revisions from all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Adams, H.D., Guardiola–Claramonte, M., Barron–Gafford, G.A., Villegas, J.C., Breshears, D.D., Zou, C.B., Troch, P.A., Huxman, T.E., 2009. Reply to Leuzinger et al.: drought-induced tree mortality temperature sensitivity requires pressing forward with best available science. Proc. Natl. Acad. Sci. 106, E107.

Adams, H.D., Zeppel, M.J., Anderegg, W.R., Hartmann, H., Landhäusser, S.M., Tissue, D. T., et al., 2017. A multi–species synthesis of physiological mechanisms in drought–induced tree mortality. Nat. Ecol. Evol. 1, 1285–1291.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., et al., 2010. A global overview of drought and heat– induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660–684.

Aschan, G., Wittmann, C., Pfanz, H., 2001. Age–dependent bark photosynthesis of aspen twigs. Trees 15, 431–437.

Aschan, G., Pfanz, H., 2003. Non–foliar photosynthesis–a strategy of additional carbon acquisition. Flora, Distrib., Funct. Ecol. Plants 198, 81–97.

Ávila, E., Herrera, A., Tezara, W., 2014. Contribution of stem CO_2 fixation to whole–plant carbon balance in nonsucculent species. Photosynthetica 52, 3–15.

Ávila-Lovera, E., Zerpa, A.J., Santiago, L.S., 2017. Stem photosynthesis and hydraulics are coordinated in desert plant species. New Phytol. 216, 1119–1129.

Bergmeyer, H.U., Bernt, E., 1974. Sucrose. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, Vol 3. Academic Press, New York, pp. 1176–1179.

Bloemen, J., Overlaet–Michiels, L., Steppe, K., 2013. Understanding plant responses to drought: how important is woody tissue photosynthesis? Acta Hortic. 991, 149–157.

Bloemen, J., Vergeynst, L.L., Overlaet-Michiels, L., Steppe, K., 2016. How important is woody tissue photosynthesis in poplar during drought stress? Trees 30, 63–72.

Brun, F., Pacile, S., Accardo, A., Kourousias, G., Dreossi, D., Mancini, L., Tromba, G., Pugliese, R., 2015. Enhanced and flexible software tools for X-ray computed tomography at the italian synchrotron radiation facility Elettra. Fundam. Inform. 141, 233–243.

Cernusak, L.A., Hutley, L.B., 2011. Stable isotopes reveal the contribution of corticular photosynthesis to growth in branches of *Eucalyptus miniata*. Plant Physiol. 155, 515–523.

Cernusak, L.A., Cheesman, A.W., 2015. The benefits of recycling: how photosynthetic bark can increase drought tolerance. N. Phytol. 208, 995–997.

Chen, X., Zhao, P., Zhao, X., Wang, Q., Ouyang, L., Larjavaara, M., et al., 2021. Involvement of stem corticular photosynthesis in hydraulic maintenance of Eucalyptus trees and its effect on leaf gas exchange. Environ. Exp. Bot. 186, 104451.

De Baerdemaeker, N.J., Salomón, R.L., De Roo, L., Steppe, K., 2017. Sugars from woody tissue photosynthesis reduce xylem vulnerability to cavitation. N. Phytol. 216, 720–727.

De Roo, L., Salomón, R.L., Oleksyn, J., Steppe, K., 2020. Woody tissue photosynthesis delays drought stress in Populus tremula trees and maintains starch reserves in branch xylem tissues. New Phytol. 228, 70–81.

Eyles, A., Pinkard, E.A., O'Grady, A.P., Worledge, D., Warren, C.R., 2009. Role of corticular photosynthesis following defoliation in Eucalyptus globulus. Plant, Cell Environ. 32, 1004–1014.

Gibson, A.C., 1983. Anatomy of photosynthetic old stems of nonsucculent dicotyledons from North American deserts. Bot. Gaz. 144, 347–362.

Hasan, M.M., Gong, L., Nie, Z.F., Li, F.P., Ahammed, G.J., Fang, X.W., 2021. ABA-induced stomatal movements in vascular plants during dehydration and rehydration. Environ. Exp. Bot. 186, 104436.

Hember, R.A., Kurz, W.A., Coops, N.C., 2017. Relationships between individual-tree mortality and water-balance variables indicate positive trends in water stress-induced tree mortality across North America. Glob. Change Biol. 23, 1691–1710.

Hölttä, T., Mencuccini, M., Nikinmaa, E., 2009. Linking phloem function to structure: analysis with a coupled xylem-phloem transport model. J. Theor. Biol. 259, 325–337.

Hubeau, M., Steppe, K., 2015. Plant–PET scans: in vivo mapping of xylem and phloem functioning. Trends Plant Sci. 20, 676–685.

Kawai, K., Minagi, K., Nakamura, T., Saiki, S.T., Yazaki, K., Ishida, A., 2022. Parenchyma underlies the interspecific variation of xylem hydraulics and carbon storage across 15 woody species on a subtropical island in Japan. Tree Physiol. 42, 337–350.

Knipfer, T., Eustis, A., Brodersen, C., Walker, A.M., McElrone, A.J., 2015. Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure. Plant, Cell Environ. 38, 1503–1513.

Konrad, W., Katul, G., Roth-Nebelsick, A., Jensen, K.H., 2018. Xylem functioning, dysfunction and repair: a physical perspective and implications for phloem transport. Tree Physiol. 39, 243–261.

Landhäusser, S.M., Chow, P.S., Dickman, L.T., Furze, M.E., Kuhlman, I., Schmid, S., et al., 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiol. 38, 1764–1778.

Liu, J., Gu, L., Yu, Y., Huang, P., Wu, Z., Zhang, Q., Qian, Y., Sun, Z., 2019. Corticular photosynthesis drives bark water uptake to refill embolized vessels in dehydrated branches of *Salix matsudana*. Plant, Cell Environ. 42, 2584–2596.

Lloret, F., Sapes, G., Rosas, T., Galiano, L., Saura-Mas, S., Sala, A., Martínez-Vilalta, J., 2018. Non-structural carbohydrate dynamics associated with drought-induced die-off in woody species of a shrubland community. Ann. Bot. 121, 1383–1396.

Loewenstein, N.J., Pallardy, S.G., 2002. Influence of a drying cycle on post-drought xylem sap abscisic acid and stomatal responses in young temperate deciduous angiosperms. New Phytol. 156, 351–361.

Losso, A., Beikircher, B., Dämon, B., Kikuta, S., Schmid, P., Mayr, S., 2017. Xylem sap surface tension may be crucial for hydraulic safety. Plant Physiol. 175, 1135–1143.

Martorell Lliteras, S., Diaz-Espejo, A., Medrano, H., Ball, M., Choat, B., 2014. Rapid hydraulic recovery in *Eucalyptus pauciflora* after drought: linkages between stem hydraulics and leaf gas exchange. Plant, Cell Environ. 37, 617–626.

McDowell, N.G., 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiol. 155, 1051–1059.

McDowell, N.G., Beerling, D.J., Breshears, D.D., Fisher, R.A., Raffa, K.F., Stitt, M., 2011. The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol. Evol. 26, 523–532.

McDowell, N.G., Coops, N.C., Beck, P.S., Chambers, J.Q., Gangodagamage, C., Hicke, J. A., et al., 2015. Global satellite monitoring of climate-induced vegetation disturbances. Trends Plant Sci. 20, 114–123.

McDowell, N.G., Sapes, G., Pivovaroff, A., Adams, H.D., Allen, C.D., Anderegg, W.R., et al., 2022. Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. Nat. Rev. Earth Environ. 3, 294–308.

Morris, H., Plavcová, L., Gorai, M., Klepsch, M.M., Kotowska, M., Schenk, H.J., Jansen, S., 2018. Vessel-associated cells in angiosperm xylem: highly specialized living cells at the symplast-apoplast boundary. Am. J. Bot. 105, 1–10.

Nardini, A., Salleo, S., Trifilò, P., Lo, Gullo, M.A., 2003. Water relations and hydraulic characteristics of three woody species co-occurring in the same habitat. Ann. For. Sci. 60, 297–305.

Nardini, A., Gascò, A., Trifilò, P., Lo Gullo, M.A., Salleo, S., 2007. Ion-mediated enhancement of xylem hydraulic conductivity is not always suppressed by the presence of Ca^{2+} in the sap. J. Exp. Bot. 58, 2609–2615.

Nardini, A., Lo Gullo, M.A., Salleo, S., 2011. Refilling embolized xylem conduits: is it a matter of phloem unloading? Plant Sci. 180, 604–611.

Nardini, A., Savi, T., Trifilò, P., Lo, Gullo, M.A., 2018. Drought stress and the recovery from xylem embolism in woody plants. In: Cánovas, F.M., Luettge, U., Matyssek, R. (Eds.), Progress in Botany. Springer, Berlin: DE, pp. 197–231.

Natale, S., La Rocca, N., Battistuzzi, M., Morosinotto, T., Nardini, A., Alboresi, A., 2023. Structure and function of bark and wood chloroplasts in a drought tolerant tree (*Fraxinus ornus* L.). Tree Physiol., tpad013 https://doi.org/10.1093/treephys/tpad013.

Neumann, M., Mues, V., Moreno, A., Hasenauer, H., Seidl, R., 2017. Climate variability drives recent tree mortality in Europe. Glob. Change Biol. 23, 4788–4797.

Nilsen, E.T., 1995. Stem photosynthesis: extent, patterns and role in plant carbon economy. In: Gartner, B. (Ed.), Plant stems: physiology and functional morphology. Academic Press, San Diego, USA, pp. 223–240.

Oparka, K.J., 1994. Plasmolysis: new insights into an old process. New Phytol. 126, 571–591.

Paganin, D., Mayo, S.C., Gureyev, T.E., Miller, P.R., Wilkins, S.W., 2002. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. J. Microsc. 206, 33–40.

Patonnier, M.P., Peltier, J.P., Marigo, G., 1999. Drought-induced increase in xylem malate and mannitol concentrations and closure of *Fraxinus excelsior* L. stomata. J. Exp. Bot. 50, 1223–1229.

Petit, G., Savi, T., Consolini, M., Anfodillo, T., Nardini, A., 2016. Interplay of growth rate and xylem plasticity for optimal coordination of carbon and hydraulic economies in *Fraxinus ornus* trees. Tree Physiol. 36, 1310–1319.

Pfanz, H., Aschan, G., 2001. The existence of bark and stem photosynthesis in woody plants and its significance for the overall carbon gain. An eco-physiological and ecological approach. Prog. Bot. 62, 477–510.

Pfanz, H., Aschan, G., Langenfeld-Heyser, R., Wittmann, C., Loose, M., 2002. Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. Naturwissenschaften 89, 147–162.

Pinheiro, J., Bates, D., Debroy, S., Sarkar, D., Core Team, R., 2016. Nlme nonlinear mixed effects models. R Package. Version 3.1–124.
Plavcova, L., Hacke, U.G., Sperry, J.S., 2011. Linking irradiance-induced changes in pit membrane ultrastructure with xylem vulnerability to cavitation. Plant, Cell Environ. 34, 501–513.
R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Saveyn, A., Steppe, K., Ubierna, N., Dawson, T.E., 2010. Woody tissue photosynthesis and its contribution to trunk growth and bud development in young plants. Plant, Cell Environ. 33, 1949–1958.
Savi, T., Andri, S., Nardini, A., 2013. Impact of different green roof layering on plant water status and drought survival. Ecol. Eng. 57, 188e196.
Savi, T., Casolo, V., Luglio, J., Bertuzzi, S., Trifilò, P., Lo Gullo, M.A., Nardini, A., 2016. Species–specific reversal of stem xylem embolism after a prolonged drought correlates to endpoint concentration of soluble sugars. Plant Physiol. Biochem. 106, 198–207.
Schenk, H.J., Espino, S., Romo, D.M., et al., 2017. Xylem surfactants introduce a new element to the cohesion-tension theory. Plant Physiol. 173, 1177–1196.
Schenk, H.J., Jansen, S., Hölttä, T., 2021. Positive pressure in xylem and its role in hydraulic function. New Phytol. 230, 27–45.
Schmitz, N., Egerton, J.J.G., Lovelock, C.E., Ball, M.C., 2012. Light-dependent maintenance of hydraulic function in mangrove branches: do xylary chloroplasts play a role in embolism repair? New Phytol. 195, 40–46.
Secchi, F., Pagliarani, C., Cavalletto, S., Petruzzellis, F., Tonel, G., Savi, T., et al., 2021. Chemical inhibition of xylem cellular activity impedes the removal of drought-induced embolisms in poplar stems-new insights from micro-CT analysis. N. Phytol. 229, 820–830.
Sevanto, S., Mcdowell, N.G., Dickman, L.T., Pangle, R., Pockman, W.T., 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ. 37, 153–161.
Steppe, K., Sterck, F., Deslauriers, A., 2015. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends Plant Sci. 20, 335–343.
Sun, Q., Yoda, K., Suzuki, M., Suzuki, H., 2003. Vascular tissue in the stem and roots of woody plants can conduct light. J. Exp. Bot. 54, 1627–1635.
Sun, Q., Yoda, K., Suzuki, H., 2005. Internal axial light conduction in the stems and roots of herbaceous plants. J. Exp. Bot. 56, 191–203.
Teskey, R.O., Saveyn, A., Steppe, K., McGuire, M.A., 2008. Origin, fate and significance of CO_2 in tree stems. New Phytol. 177, 17–32.
Thonglim, A., Delzon, S., Larter, M., Karami, O., Rahimi, A., Offringa, R., et al., 2021. Intervessel pit membrane thickness best explains variation in embolism resistance amongst stems of *Arabidopsis thaliana* accessions. Ann. Bot. 128, 171–182.
Tixier, A., Orozco, J., Roxas, A.A., Earles, J.M., Zwieniecki, M.A., 2018. Diurnal variation in nonstructural carbohydrate storage in trees: remobilization and vertical mixing. Plant Physiol. 178, 1602–1613.
Tomasella, M., Häberle, K.H., Nardini, A., Hesse, B., Machlet, A., Matyssek, R., 2017. Post-drought hydraulic recovery is accompanied by non-structural carbohydrate depletion in the stem wood of Norway spruce saplings. Sci. Rep. 7, 14308.
Tomasella, M., Casolo, V., Aichner, N., Petruzzellis, F., Savi, T., Trifilò, P., Nardini, A., 2019a. Non-structural carbohydrate and hydraulic dynamics during drought and recovery in *Fraxinus ornus* and *Ostrya carpinifolia* saplings. Plant Physiol. Biochem. 145, 1–9.
Tomasella, M., Petrussa, E., Petruzzellis, F., Nardini, A., Casolo, V., 2019b. The possible role of non-structural carbohydrates in the regulation of tree hydraulics. Int. J. Mol. Sci. 21, 144.
Tomasella, M., Casolo, V., Natale, S., Petruzzellis, F., Kofler, W., Beikircher, B., Stefan Mayr, Nardini, A., 2021. Shade-induced reduction of stem nonstructural carbohydrates increases xylem vulnerability to embolism and impedes hydraulic recovery in Populus nigra. New Phytol. 231, 108–121.
Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni Stefano, Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci. Rep. 5, 1–12.
Torres-Ruiz, J.M., Jansen, S., Choat, B., McElrone, A.J., Cochard, H., Brodribb, et al., 2015. Direct X-ray microtomography observation confirms the induction of embolism upon xylem cutting under tension. Plant Physiol. 167, 40–43.
Trifilò, P., Raimondo, F., Lo Gullo, M.A., Barbera, P.M., Salleo, S., Nardini, A., 2014. Relax and refill: xylem rehydration prior to hydraulic measurements favours embolism repair in stems and generates artificially low PLC values. Plant, Cell Environ. 37, 2491–2499.
Trifilò, P., Casolo, V., Raimondo, F., Petrussa, E., Boscutti, F., Lo Gullo, M.A., Nardini, A., 2017. Effects of prolonged drought on stem non-structural carbohydrates content and post-drought hydraulic recovery in *Laurus nobilis* L.: the possible link between carbon starvation and hydraulic failure. Plant Physiol. Biochem. 120, 232–241.
Trifilò, P., Natale, S., Gargiulo, S., Abate, E., Casolo, V., Nardini, A., 2021. Stem photosynthesis affects hydraulic resilience in the deciduous *Populus alba* but not in the evergreen *Laurus nobilis*. Water 13, 2911.
Vandegehuchte, M.W., Bloemen, J., Vergeynst, L.L., Steppe, K., 2015. Woody tissue photosynthesis in trees: salve on the wounds of drought? New Phytol. 208, 998–1002.
Wang, S., Xie, Y., Niu, S., Lin, L., Liu, C., Zhou, Y.S., Wang, Z.L., 2014. Maximum surface charge density for triboelectric nanogenerators achieved by ionized-air injection: methodology and theoretical understanding. Adv. Mater. 26, 6720–6728.
Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313.
Wheeler, J.K., Huggett, B.A., Tofte, A.N., Rockwell, F.E., Holbrook, N.M., 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant, Cell Environ. 36, 1938–1949.
Wittmann, C., Pfanz, H., 2008. General trait relationships in stems: a study on the performance and interrelationships of several functional and structural parameters involved in corticular photosynthesis. Physiol. Plant. 134, 636–648.
Yemm, E.W., Willis, A., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508.