



Structural and anatomical responses of *Pinus sylvestris* and *Tilia platyphyllos* seedlings exposed to water shortage

Natasa Kiorapostolou¹ · Lucía Galiano-Pérez² · Georg von Arx^{2,3} · Arthur Gessler² · Gaii Petit¹

Received: 26 January 2018 / Accepted: 2 May 2018 / Published online: 9 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Key message Under drought, *P. sylvestris* produces more needle vs. xylem biomass to prevent C reserve imbalances, whereas *T. platyphyllos* invests in water transport efficiency, producing wider vessels at the stem apex.

Abstract Phenomena of tree decline and mortality are increasing worldwide as a consequence of the higher temperatures accompanying drought events. Studying changes in biomass allocation and xylem anatomy may shed light on the relative importance that C and water impairment have during drought, and can help to better understand how plants will respond to future droughts. We measured the dry weight of the leaf, aboveground and belowground xylem biomass in tree seedlings of the drought-avoidant *Pinus sylvestris* and drought-tolerant *Tilia platyphyllos* exposed to different intensities of water shortage. Moreover, the area of vessels was measured at three positions along the stem. In *P. sylvestris*, we found no differences in total biomass across treatments, but a preferential allocation to needle mass under drought, while there were no differences in xylem anatomy. *Tilia platyphyllos* under ambient and mild drought increased leaf and total xylem biomass according to an isometric pattern, whereas the largest vessels near the stem apex were found in seedlings under severe drought. Our results suggest a categorisation of the two species regarding the coordination of carbon and hydraulic economies. *Pinus sylvestris* invests relatively more into leaf biomass to increase photosynthesis and thus decreases the risk of carbon starvation, while *T. platyphyllos* invests more into hydraulic efficiency to decrease the risks of embolisation.

Keywords *Pinus sylvestris* · *Tilia platyphyllos* · Drought · Biomass allocation · Xylem anatomy · Isohydry

Introduction

The average global temperature has already increased by 0.85 °C since 1880 (IPCC 2014), and even higher temperatures are expected in the future, implying warmer summers and winters, thus longer growing seasons (Menzel and Fabian 1999), and possibly the occurrence of more frequent

extreme drought events in various areas of the Earth due to more erratic precipitation regimes (IPCC 2014). Within such a perspective, species are expected to adopt acclimation and/or adaptation strategies to withstand the new climatic conditions (Jump and Penuelas 2005; De Micco and Aronne 2012). In recent years, however, higher rates of tree mortality and forest dieback have been reported at global scale after severe drought events (Allen et al. 2010). Although we still lack a clear understanding on the ultimate mechanism leading to tree decline and mortality under drought (Mitchell et al. 2013), the coordination between carbon and hydraulic economies is emerging as a fundamental requirement for plant survival (Mencuccini 2014; Mitchell et al. 2014; Petit et al. 2016; Sterck and Zweifel 2016).

Plants are distributed across a continuum from drought-avoidant to drought-tolerant species, and according to their stomatal regulation under different soil water potentials (Martínez-Vilalta and Garcia-Forner 2017), from isohydric to anisohydric species. Relatively isohydric species close stomata at a defined minimum leaf water potential (Ψ_{L_MIN})

Communicated by Y. Sano.

✉ Natasa Kiorapostolou
natasa.kiorapostolou@phd.unipd.it

¹ Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy

² Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

³ Climatic Change and Climate Impacts, Institute for Environmental Sciences, 66 Blvd Carl Vogt, 1205 Geneva, Switzerland

set at relatively moderate negative pressures, while relatively anisohydric species are able to lower their Ψ_{L_MIN} to maintain a certain degree of leaf gas exchange even under water deficits (Attia et al. 2015). These different modes of stomatal control are supposedly linked to different survival strategies under drought. Under this scenario, relatively isohydric species would preserve the transport system from xylem tensions potentially triggering conduit embolisation. This implies that these species most likely rely upon stored carbon reserves to sustain the respiratory metabolism under drought, and may, therefore, die of carbon starvation after substantial depletion of carbon resources during prolonged drought (McDowell et al. 2008). On the contrary, relatively anisohydric species probably need to keep water transport efficient to sustain leaf transpiration and carbon assimilation even under drought. Thus, anisohydric species would expose their xylem system to the risk of hydraulic failure because of excessive cavitation events triggered by the extreme high tensions that could develop under very intense water deficits, irrespective of their duration (Attia et al. 2015). These species do not necessarily rely upon stored reserves to survive during drought events (McDowell 2011).

Plants have been shown to allocate their functional/structural traits following allometric relationships that commonly follow power scaling functions:

$$Y = a \cdot X^b, \quad (1)$$

where Y and X are functional/structural traits, a the allometric constant and b the scaling exponent. According to optimality principles (West et al. 1999), these relationships between traits would imply a substantial convergence towards a common scaling exponent (b), reflecting the necessary balance between structures and functions to maintain a positive carbon balance (Anfodillo et al. 2016). On the contrary, differences in the allometric constant (a) would reflect modifications in the absolute proportion between traits (e.g. between leaf mass and xylem mass) that may emerge in different environmental conditions (Weiner 2004). In this scheme, Anfodillo et al. (2016) suggested that the death of a plant would be related to non-reversible departures from the general scaling between traits (similar scaling exponent b), with high and low thresholds of the allometric constant (a) representing the limits to plant functionality. A sustainable balance of carbon production vs. consumption is reached when a given leaf mass provides at least the necessary carbon resources to sustain the maintenance cost of the living tissues in a given xylem mass, whereas the invested biomass to below- and aboveground xylem must guarantee an efficient soil water absorption and transport that meet the leaf transpiration requirements.

Water is transported under a negative pressure gradient through the elongated dead cells of the xylem, which can be compared to capillary tubes and, therefore, produce a

frictional resistance to flow inversely proportional to the fourth power of their diameter (Hagen-Poiseuille law, Tyree and Ewer 1991). On the contrary, more adhesive forces for a unit of water volume can develop on smaller xylem conduits that can better cope with the metastable status of tensile water (Hacke et al. 2017). Therefore, xylem anatomy is strongly related to the total hydraulic safety and efficiency (Hacke and Sperry 2001), and thus to the plant performance under different soil water conditions. A common pattern often found in xylem anatomy studies is the production of narrower and hydraulically safer vessels in drier environments (von Arx et al. 2012; Pfautsch et al. 2016; Larter et al. 2017), with the reduced growth rate being the “cost” of their reduced hydraulic efficiency (Wheeler et al. 2005). Instead, other empirical evidence demonstrates that plant architecture is organised with conduit lumen areas widening from the stem apex towards the base according to a scaling pattern that is very similar between species in different environments (Anfodillo et al. 2013). The hydraulic consequence of such a pattern is that most of the resistance is concentrated within a short distance from the apex (Yang and Tyree 1993; Becker et al. 2000; Petit and Anfodillo 2009), thus making the apical anatomical features of particular importance for the whole plant conductance (Petit et al. 2011; Prendin et al. 2018a) and also for hydraulic vulnerability (Prendin et al. 2018b).

In this study, seedlings of two species (*Pinus sylvestris* L. and *Tilia platyphyllos* Scop.) were subjected to two water shortage intensities to test their performance under drought conditions in terms of biomass allocation to leaf, stem and root tissues, and also to evaluate the degree of plastic adjustment at the xylem anatomical level. As the species have been shown to differ in terms of hydric behaviour, with *P. sylvestris* to be relatively isohydric (Irvine et al. 1998) and *T. platyphyllos* relatively anisohydric (Leuzinger et al. 2005; Galiano et al. 2017), we hypothesised that *P. sylvestris* will associate more needles with a given xylem biomass by prioritising allocation to needle biomass to prevent C reserve imbalances (e.g. C reserve depletion), while *T. platyphyllos* will maintain the structural balance between leaf and xylem biomass but will adjust its xylem anatomy to maintain an efficient water transport under drought.

Materials and methods

Plant materials and experimental design

Our two target species were selected based on different responses to drought conditions: *P. sylvestris* L. (PS) has a strong stomatal control and minimises stomatal conductance under drought, even if this can produce intense depletion of stored carbon reserves potentially leading to mortality events

(Aguadé et al. 2015). Instead, *T. platyphyllos* Scop. (TP), a deciduous angiosperm with diffuse to semi-ring porosity, has a less strong stomatal control of transpiration and has been found to recover well after drought relief (Leuzinger et al. 2005).

Three-year-old seedlings of the two species ($N=36$), with a mean height of 28.16 ± 0.86 cm (mean \pm se) for *P. sylvestris* and 30.59 ± 1.60 cm for *T. platyphyllos*, were planted in 3.5-L pots in the beginning of May 2014 in the greenhouse of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL ($47^{\circ}21'37''N$, $08^{\circ}27'21''E$; 500 m a.s.l.). The seedlings were planted before leaf development in *T. platyphyllos*. Greenhouse temperature ranged from 9 to 37 °C and air humidity from 22 to 74%. Artificial illumination was applied (Master Green Power CG T 400 W Mogul 1SL/12. Phillips Lighting Holding B.V., Eindhoven, The Netherlands) simulating the mean summer photoperiod of Swiss latitudes (~ 15 h light). During the acclimation period in the greenhouse, all seedlings were watered every second day to field capacity. Six pots from each of the two species were allocated to each of the three watering treatments paying attention that size variability between treatments was similar. The treatments started on 14th July in *T. platyphyllos* and 28th July in *P. sylvestris*, when seedlings showed vigorous conditions and leaves fully developed, and were applied continuously for approximately 2 months (until 14th September in *T. Platyphyllos* and 28th September in *P. sylvestris*), and were:

- Control (C): normal watering with soil volumetric water content (VWC) maintained at field capacity $\sim 23\%$.
- Moderate drought (MD): watering adjusted to maintain VWC at 15%, which equalled the soil water potential (Ψ_{SOIL}) ~ -0.12 MPa.
- Severe drought (SD): watering adjusted to maintain VWC at 8%, which equalled $\Psi_{\text{SOIL}} \sim -1.3$ MPa.

Measurements

At the end of the experiment, each plant was extracted from the pot and the root system cleaned from soil residues. The height of the plants was measured, then plants were divided into three different organs (leaves/needles, aboveground stem and branches including the bark, and belowground roots), which were carefully separated and dried in an oven at 60 °C for 72 h. The dry mass of all leaves/needles (LM), aboveground stem and branches (AM), and roots (RM) were obtained using a balance (Acculab ALC-1100.2) to the nearest 0.1 g. Total leaf area (LA_{TOT}) was measured in scanned images with ROXAS v2.1 (von Arx and Dietz 2005; von Arx and Carrer 2014). Total biomass (BM_{TOT}), the ratio

LM:(AM+RM), and specific leaf area (SLA) were later computed.

Stem segments were then collected at three different heights (stem base, 20 cm from the apex, 2 cm from the apex) from each of the six seedlings per species and watering treatments (total samples = 108). Sample preparation and anatomical analyses followed the guidelines proposed by von Arx et al. (2016). Micro-sections were created using a rotary microtome LEICA RM 2245 (Leica Biosystems, Nussloch, Germany) at 15 μm thickness, stained with a solution of safranin and astra blue (1 and 0.5% in distilled water, respectively), and permanently fixed on glasses with Eukitt (BiOptica, Milan, Italy). Overlapping images (around 25%) of the entire cross section of each sample were taken with a digital camera mounted on a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan), and then stitched with PTGui (New House Internet Services B.V., Rotterdam, The Netherlands). Stitched images were then analysed with ROXAS v2.1 (von Arx and Dietz 2005; von Arx and Carrer 2014) for the automatic measurement of the lumen area (CA) of all the vessels in the outmost ring, and their frequency distribution in CA size classes of 20 μm^2 for *P. sylvestris* and 30 μm^2 for *T. platyphyllos*.

Statistical analysis

LM, AM, RM, BM_{TOT} , LA_{TOT} , SLA, and LM:(AM+RM) were all normally distributed. One-way ANOVA with Tukey post hoc comparisons were used to test differences across watering treatments (C, MD, SD). To test the differences of CA size classes in the apical part of the branches (2 cm from the apex), a Kruskal–Wallis test was performed for the non-parametric data of the six largest CA size classes. All statistical analyses were conducted with standard methods (Zar 1999) using the software IBM SPSS Version 21 and Minitab 18.

Results

Pinus sylvestris

The overall structure of *P. sylvestris* seedlings did not seem to be changed at the end of the experiment. Mean final plant height (29.15 ± 0.93 cm) was not significantly different from the initial and we did not find significant differences in the height of the plants across treatments ($p > 0.05$). Moreover, the seedlings did not show any significant difference in BM_{TOT} , LM, AM, and RM across watering treatments (Fig. 1a; Table 1). However, when we compared the allocation to leaf vs. xylem biomass, we found that the individuals exposed to severe drought (SD) showed a preferential

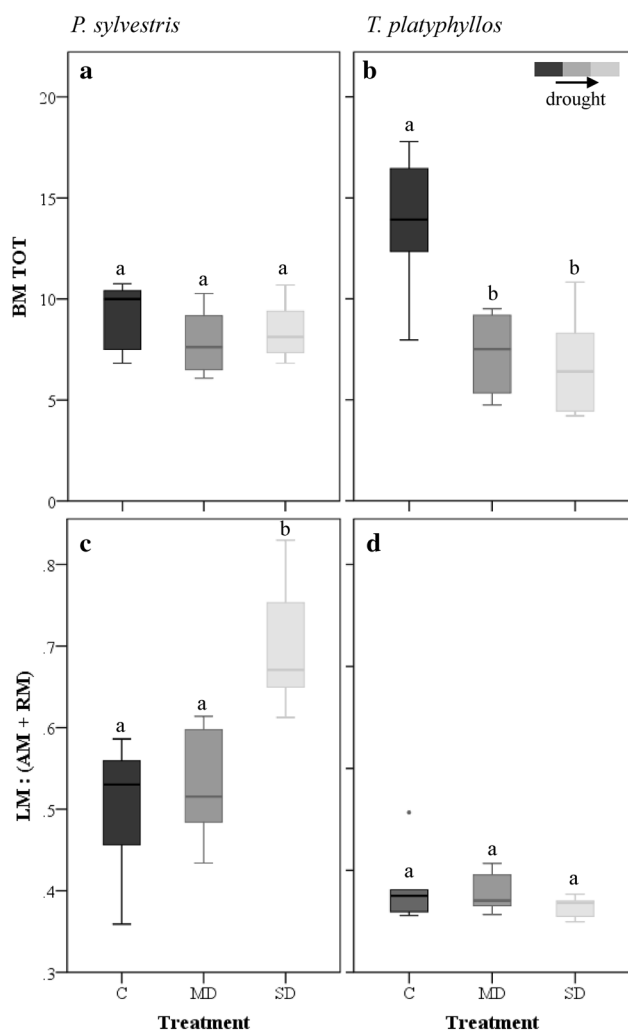


Fig. 1 Total biomass (BM_{TOT}) (g) for *P. sylvestris* (PS) (a) and *T. platyphyllos* (TP) (b), and the ratio leaf mass/stem and root mass [LM:(AM+RM)] for PS (c) and TP (d) across control (C), moderate drought (MD), and severe drought (SD) treatments. Letters above the bars show the grouping with a p value < 0.05 performed with Tukey post hoc tests

allocation to the needle mass, resulting in a significantly higher ratio of LM:(AM+RM) (Figs. 1c, 2).

At the wood anatomy level, the distribution of tracheids in classes of lumen areas (CA) did not change much among treatments for stem distance from the apex (Fig. 3). Mean cell lumen areas (MCA) increased from the stem apex to the base in all watering treatments (the so-called conduit widening) (Fig. 3, reference lines).

Tilia platyphyllos

In *T. platyphyllos* seedlings, the application of water shortage treatments affected the overall plant structure and xylem anatomy. The mean final height (31.24 ± 1.55 cm) did not differ significantly from the initial and across treatments ($p > 0.05$). Instead, the drought treatments significantly and negatively affected the total biomass production. Indeed, the BM_{TOT} was significantly lower (by approximately 50%) under both moderate (MD) and severe drought (SD) treatments compared to the control (C) (Table 1; Fig. 1b). However, the ratio LM:(AM+RM) did not differ across watering treatments (Fig. 1d, $b = 0.89$, 95% CI = 0.7197, 0.9936). LA_{TOT} tended to be lower and SLA higher with increasing drought but only LA_{TOT} was significantly different in the severe drought (SD) relative to the control (C) (Table 1).

At the wood anatomy level, we found some differences among watering treatments (Fig. 3). In particular, the analysis of the anatomical structure at 2 cm from the stem apex revealed that seedlings exposed to the severe drought (SD) produced more vessels in the six widest CA classes (Kruskal–Wallis, $\chi^2 = 39.5$, $p < 0.001$) (Fig. 3). However, the mean cell lumen area (MCA) at the different positions along the stem did not vary much between watering treatments, but slightly increased from the apex downwards (Fig. 3, reference lines).

Table 1 Mean values (standard error) for each single variable across control (C), moderate drought (MD) and severe drought (SD) treatments in *P. sylvestris* (PS, upper panel) and *T. platyphyllos* (TP, lower panel)

	LM (g)	AM (g)	RM (g)	LA _{TOT} (cm ²)	SLA (cm ² /g)
PS					
C	3.06 (0.23)a	2.50 (0.28)a	3.69 (0.34)a	132.99 (100)a	43.53 (107)a
MD	2.71 (0.25)a	2.13 (0.12)a	3.03 (0.37)a	110.76 (83) a	41.19 (92)a
SD	3.44 (0.22)a	2.15 (0.17)a	2.83 (0.21)a	151.70 (95)a	44.17 (63)a
TP					
C	1.85 (0.19)a	3.23 (0.33)a	8.66 (2.73)a	509.32 (96)a	300.95 (39)a
MD	0.95 (0.09)b	2.25 (0.30)a	4.11 (1.29)b	276.58 (26)ab	331.83 (45)a
SD	0.77 (0.12)b	2.05 (0.41)a	3.95 (1.46)b	255.26 (69)b	389.93 (106)a

Letters (a, b) show significant grouping differences

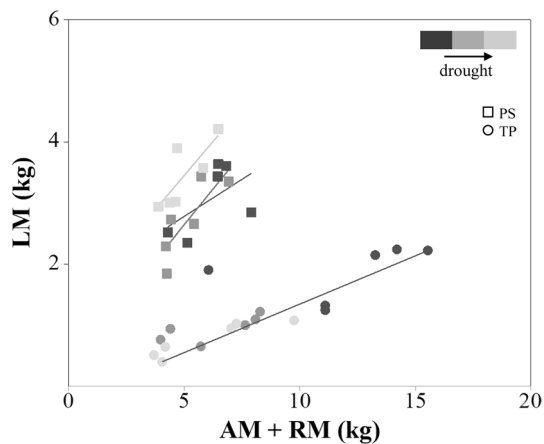


Fig. 2 Leaf biomass (g) against combined stem and root biomass (g) for *P. sylvestris* (PS, squares) and *T. platyphyllos* (TP, circles) across watering treatments (C, MD, SD). Statistics for PS: (C) $y = 1.1444x^{0.5376}$, $R^2 = 0.3803$, $p = 0.26$, (MD) $y = 0.558x^{0.96}$, $R^2 = 0.6726$, $p = 0.042^*$, (SD) $y = 1.2006x^{0.6554}$, $R^2 = 0.6539$, $p = 0.053$. Statistics for TP: $y = 0.1791x^{0.8933}$, $R^2 = 0.7071$

Discussion

Our greenhouse experiment revealed that the seedlings of the relatively isohydric *P. sylvestris* and the relatively anisohydric *T. platyphyllos* responded differently to drought exposure in terms of biomass allocation to the different organs and of wood anatomy.

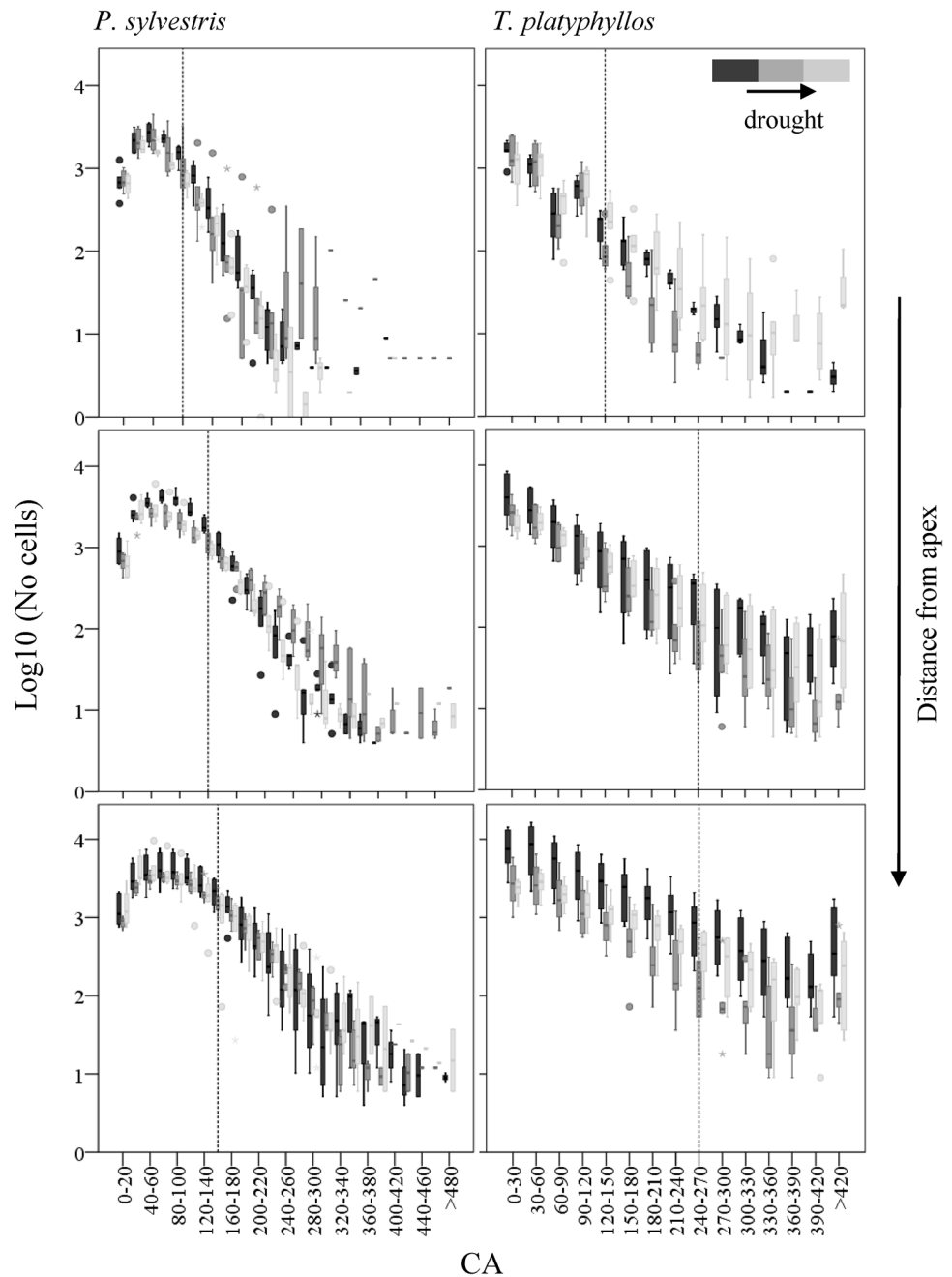
In our *P. sylvestris* seedlings, we found little but not significant modifications in the overall plant biomass allocation and xylem anatomy. Because of the restricted size variation between seedlings, we could not assess a clear scaling relationship between LM and AM + RM explaining how LM must be adjusted to sustain the cost of maintenance associated with the living tissues in the xylem (Anfodillo et al. 2016). However, our results suggest that drought slightly affected the mass balance between C-producer (LM) and C-consumer (AM + RM) tissues (Figs. 1c, 2). Whatever the process mostly affected under drought was, either the accumulation of NSC in the needles or the slower production of fine roots (Galiano et al. 2017), our results suggest that drought-exposed pine seedlings modified the balance between LM and AM + RM in a way that a higher needle mass was associated with a given mass of tissues consuming carbon. A higher NSC concentration in the leaves (see also Galiano et al. 2017) may have provided the necessary osmoregulation to keep stomata open also under drier conditions. Alternatively, such a result is consistent with a recent hypothesis (Anfodillo et al. 2016) according to which species that cannot lower their leaf water potential (Ψ_L) enough to maintain the stomata open during reduced soil water (like *P. sylvestris*), must provide the carbon required for respiration by increasing the proportion of leaf/needle mass (see

also Petit et al. 2016). Regarding the anatomical characteristics, we did not find any substantial difference in the size of xylem conduits along the stem across the three watering treatments. However, it is likely that cambial phenology was almost complete at the time of the experiment, and we cannot exclude the possibility that *P. sylvestris* modifies also its xylem anatomy when drought occurs before or during the intense cambial activity at the beginning of the season.

In *T. platyphyllos* seedlings, the two drought treatments negatively affected total biomass production (Fig. 1b), in agreement with other studies referring to other species (e.g. Maseda and Fernández 2016). We found a nearly isometric relationship (i.e. a power scaling with exponent b not significantly different from $b = 1$) between LM and AM + RM irrespective of the watering treatment (Fig. 2). This means that drought did not affect the proportionality between leaf biomass (C-producer) and biomass of aboveground plus belowground xylem (C-consumer) (Figs. 1d, 2). Therefore, different from *P. sylvestris*, the maintenance cost of a given unit of xylem biomass in *T. platyphyllos* is sustained by a given unit of leaf biomass irrespective of soil conditions. The isometric relationship between LM and AM + RM suggests maintenance of the functional balance between leaf photosynthesis and xylem water transport. The fact that *T. platyphyllos* maintained the same proportion between different functional tissues (leaf and xylem biomass) also under drought conditions (Fig. 1d) suggests that this species has a rather conservative strategy in the differential allocation between functional tissues (Reich et al. 2008; Anfodillo et al. 2016). Under drought this implies an adjustment in Ψ_L and/or an increase in total xylem conductance to maintain leaf transpiration. On the one hand, *T. platyphyllos* reduced Ψ_L slightly more under drought than *P. sylvestris* (Galiano et al. 2017), thus being relatively more anisohydric. On the other hand, we found an increase in the lumen area (CA) of apical conduits under severe drought (SD). Such a result strongly suggests an increase in xylem conductance in response to drought (see also Petit et al. 2016), in agreement with previous reports revealing the fundamental importance of the xylem anatomy close to the apex for the efficiency of the whole hydraulic transport system (Petit et al. 2011; Prendin et al. 2018a).

Taken together, *T. platyphyllos* has a lower leaf biomass associated with a given xylem biomass than *P. sylvestris* (i.e. lower y-intercept in Fig. 2). We can assume that the C-cost associated with tissue respiration is at least similar between species, since the amount of living, and thus respiring, xylem parenchyma is certainly not higher in *P. sylvestris* than *T. platyphyllos* (Schweiggruber 1990). Therefore, our results would suggest that a unit of leaf mass in *T. platyphyllos* must provide more carbon than *P. sylvestris* needles (Galiano et al. 2017), i.e. they must be photosynthetically more efficient because the amount of assimilated carbon per leaf biomass

Fig. 3 Size classes of cell lumen areas (CA) for *P. sylvestris* (left panels) and *T. platyphyllos* (right panels) at different distances from the stem apex (2 cm upper panels, 20 cm middle panels and ~ 40 cm lower panels) and across watering treatments (C, MD and SD). The reference lines show the mean CA of each distance from the apex and widening from the apex towards the base



required for the maintenance costs of a given xylem biomass (leaf-based maintenance costs) is higher in *T. platyphyllos* as it has higher SLA values (Table 1). In agreement with this, *P. sylvestris* showed relatively stronger stomatal control of water losses by transpiration than *T. platyphyllos* (Galiano et al. 2017), with Ψ_{L_MIN} commonly being less negative, suggesting an earlier interruption of leaf gas exchange and thus photosynthesis under water-deficit conditions (Irvine et al. 1998). In addition, we can speculate that the seedlings of *T. platyphyllos* acclimated to soil drought by reducing the investment in growth because of the high leaf-based maintenance costs (low y-intercept in Fig. 2). This would

imply that leaf transpiration and photosynthesis are also maintained under water shortage. Consistently, we found a slightly higher number of vessels in the largest classes of lumen area (CA) near the apex under severe drought (SD). Since the xylem cell conductance scales to the second power of its area (according to Hagen–Poiseuille: Tyree and Ewers 1991), and apical conduits represent the hydraulic bottleneck of the entire xylem transport system (Petit et al. 2011), such a result would suggest an attempt to increase the overall conductive capacity of the xylem transport system by investing the least carbon into the new xylem biomass. This result is highly relevant, as it provides evidence that future

acclimation of a species that tolerates drought implies prioritised investment in xylem efficiency vs. safety. This is supported by recent investigations (Petit et al. 2016) reporting that under reduced soil water availability, *Fraxinus ornus* L. trees reduce the C costs associated with growth while producing wider vessels to maintain an efficient leaf specific conductance. However, this finding is in contrast to the literature reporting narrower and safer conduits in drier environments (e.g. Wheeler et al. 2005; Lens et al. 2007; von Arx et al. 2012; Pfautsch et al. 2016; Larter et al. 2017). In this context, it is worth highlighting that most studies simply ignored the existence of a substantial axial variation of vessel/tracheid lumen area from the apex downwards (Petit and Anfodillo 2011a, b), and such a pattern must be filtered out through a proper standardisation procedure when assessing the effect of any environmental factor on wood anatomical properties (Lechthaler et al. 2018).

Conclusion

Based on our results, *P. sylvestris* seems to respond morphologically and *T. platyphyllos* anatomically to ultimately adapt their carbon and hydraulic economies to drought conditions.

Pinus sylvestris has less xylem biomass associated with a unit of needle/leaf biomass than *T. platyphyllos* and, therefore, it has less corresponding living tissues. The species prioritises allocation to needle vs. xylem biomass under drought, likely to enhance the total assimilation per time unit of stomata opening, while maintaining safe conditions against the risk of xylem cavitation. However, such a strategy may lead in the long run to carbon reserve depletion and ultimately to death from carbon starvation.

Instead, the high leaf-based maintenance costs of *T. platyphyllos* impose a strategy to maintain a more continuous carbon assimilation and consequently transpiration, also under drier conditions. Therefore, the hydraulic efficiency (i.e. conductance) of the xylem transport system is prioritised over safety, exposing the xylem to lower water potential under drought, even if this could lead to widespread cavitation events.

Author contribution statement LGP and AG conceived and designed the experiment. GP and GvA collected the data. NK and GP analysed the data and wrote a first draft of the paper, which was revised and improved by all co-authors.

Acknowledgements We warmly thank Cristian Di Norscia for his support in lab work. This work was supported by: GP received financial support from the University of Padova (60A08-2852/15 and DOR1611588/16); GvA was supported by a Grant from the Swiss State Secretariat for Education, Research and Innovation SERI (SBFI C12.0100).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Aguadé D, Poyatos R, Gómez M, Oliva J, Martínez-Vilalta J (2015) The role of defoliation and root rot pathogen infection in driving the mode of drought-related physiological decline in Scots pine (*Pinus sylvestris* L.). *Tree Physiol* 35:229–242
- Allen CD, Macalady AK, Chenchouni H et al (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684
- Anfodillo T, Petit G, Crivellaro A (2013) Axial conduit widening in woody species: a still neglected anatomical pattern. *IAWA J* 34:352–364
- Anfodillo T, Petit G, Sterck F, Lechthaler S, Olson ME (2016) Allometric trajectories and “stress”: a quantitative approach. *Front Plant Sci* 7:1681–1687
- Attia Z, Domec JC, Oren R, Way DA, Moshelion M (2015) Growth and physiological responses of isohydric and anisohydric poplars to drought. *J Exp Bot* 66:4373–4381
- Becker P, Gribben RJ, Lim CM (2000) Tapered conduits can buffer hydraulic conductance from path-length effects. *Tree Physiol* 20:965–967
- De Micco V, Aronne G (2012) Morpho-anatomical traits for plant adaptation to drought. In: *Plant responses to drought stress*. Springer, Heidelberg, pp 37–62
- Galiano L, Timofeeva G, Saurer M, Siegwolf R, Martínez-Vilalta J, Hommel R, Gessler A (2017) The fate of recently fixed carbon after drought release: towards unravelling C storage regulation in *Tilia platyphyllos* and *Pinus sylvestris*. *Plant Cell Environ* 40:1711–1724
- Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. *Perspect Plant Ecol Evol Syst* 4:97–115
- Hacke UG, Spicer R, Schreiber SG, Plavcová L (2017) An ecophysiological and developmental perspective on variation in vessel diameter. *Plant Cell Environ* 40:831–845
- IPCC (2014) *Climate change 2014—impacts, adaptation, and vulnerability: regional aspects*. Cambridge University Press, Cambridge
- Irvine J, Perks MP, Magnani F, Grace J (1998) The response of *Pinus sylvestris* to drought: stomatal control of transpiration and hydraulic conductance. *Tree Physiol* 18:393–402
- Jump AS, Penuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol Lett* 8:1010–1020
- Larter M, Pfautsch S, Domec JC, Trueba S, Nagalingum N, Delzon S (2017) Aridity drove the evolution of extreme embolism resistance and the radiation of conifer genus *Callitris*. *New Phytol* 215:97–112
- Lens F, Baas P, Jansen S, Smets E (2007) A search for phylogenetically informative wood characters within Lecythidaceae sl. *Am J Bot* 94:483–502
- Lechthaler S, Tarryn LT, Gelmini Y, Pirotti F, Anfodillo T, Adams MA, Petit G (2018) Standardizing xylem anatomical traits to disentangle environmental information from axial trends. *Tree Physiol* (under review)
- Leuzinger S, Zotz G, Asshoff R, Körner C (2005) Responses of deciduous forest trees to severe drought in Central Europe. *Tree Physiol* 25:641–650
- Martínez-Vilalta J, García-Förner N (2017) Water potential regulation, stomatal behaviour and hydraulic transport under drought:

- deconstructing the iso/anisohydric concept. *Plant Cell Environ* 40:962–976
- Maseda PH, Fernández RJ (2016) Growth potential limits drought morphological plasticity in seedlings from six *Eucalyptus provenances*. *Tree Physiol* 36:243–251
- McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol* 155:1051–1059
- McDowell N, Pockman WT, Allen CD et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739
- Mencuccini M (2014) Temporal scales for the coordination of tree carbon and water economies during droughts. *Tree Physiol* 34:439–442
- Menzel A, Fabian P (1999) Growing season extended in Europe. *Nature* 397:659
- Mitchell PJ, O’Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytol* 197:862–872
- Mitchell PJ, O’Grady AP, Tissue DT, Worledge D, Pinkard EA (2014) Co-ordination of growth, gas exchange and hydraulics define the carbon safety margin in tree species with contrasting drought strategies. *Tree Physiol* 34:443–458
- Petit G, Anfodillo T (2009) Plant physiology in theory and practice: an analysis of the WBE model for vascular plants. *J Theor Biol* 259:1–4
- Petit G, Anfodillo T (2011a) Comment on “The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency” by Meinzer et al. (2010). *Oecologia* 165:271–274
- Petit G, Anfodillo T, Carraro V, Grani F, Carrer M (2011b) Hydraulic constraints limit height growth in trees at high altitude. *New Phytol* 189:241–252
- 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
- Pfautsch S, Harbusch M, Wesolowski A, Smith R, Macfarlane C, Tjoelker MG, Reich PB, Adams MA (2016) Climate determines vascular traits in the ecologically diverse genus *Eucalyptus*. *Ecol Lett* 19:240–248
- Prendin AL, Petit G, Fonti P, Rixen C, Dawes MA, von Arx G (2018a) Axial xylem architecture of *Larix decidua* exposed to CO₂ enrichment and soil warming at the treeline. *Funct Ecol* 32:273–287
- Prendin AL, Mayr S, Beikircher B, von Arx G, Petit G (2018b) Xylem anatomical adjustments prioritize hydraulic efficiency over safety as Norway spruce trees grow taller. *Tree Physiol* (**under review**)
- Reich PB, Tjoelker MG, Pregitzer KS, Wright IJ, Oleksyn J, Machado JL (2008) Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecol Lett* 11:793–801
- Schweingruber FH (1990) Microscopic wood anatomy: structural variability of stems and twigs in recent and subfossil woods from Central Europe. Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland
- Sterck F, Zweifel R (2016) Trees maintain a similar conductance per leaf area through integrated responses in growth, allocation, architecture and anatomy. *Tree Physiol* 36:1307–1309
- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360
- von Arx G, Carrer M (2014) ROXAS—a new tool to build centuries-long tracheid-lumen chronologies in conifers. *Dendrochronologia* 32:290–293
- von Arx G, Dietz H (2005) Automated image analysis of annual rings in the roots of perennial forbs. *Int J Plant Sci* 166:723–732
- von Arx G, Archer SR, Hughes MK (2012) Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. *Ann Bot* 109:1091–1100
- von Arx G, Crivellaro A, Prendin AL, Čufar K, Carrer M (2016) Quantitative wood anatomy—practical guidelines. *Front Plant Sci* 7:781
- Weiner J (2004) Allocation, plasticity and allometry in plants. *Perspect Plant Ecol Syst* 6:207–215
- West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of plant vascular systems. *Nature* 400:664–667
- Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ* 28:800–812
- Yang S, Tyree MT (1993) Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. *Tree Physiol* 12:231–242
- Zar JH (1999) *Biostatistical analysis*. Pearson Education, New Delhi