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Research

Hydraulic-stomatal coordination in tree seedlings: tight correlation across environments and ontogeny in *Acer pseudoplatanus*

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Summary

- Hydraulic conductance is recognized as a major determinant of gas exchange and productivity. However, whether this also applies to seedlings, a critically important stage for vegetation regeneration, has been largely unknown.
- We analyzed the hydraulic and stomatal conductance of leaves and shoots for 6-wk-old *Acer pseudoplatanus* seedlings emerging in different lowland and treeline habitats and under glasshouse conditions, respectively, as well as on 9-, 15- and 18-wk-old plants, and related findings to leaf and xylem anatomical traits.
- Treeline seedlings had higher leaf area-specific shoot hydraulic conductance ($K_{\text{shoot-L}}$), and stomatal conductance (g_s), associated with wider xylem conduits, lower leaf area and higher stomatal density than lowland and glasshouse-grown plants. Across the first 18 wk of development, seedlings increased four-fold in absolute shoot hydraulic conductance (K_{shoot}) and declined by half in $K_{\text{shoot-L}}$, with correlated shifts in xylem and leaf anatomy. Distal leaves had higher leaf hydraulic conductance (K_{leaf}) and g_s compared to basal leaves.
- Seedlings show strong variation across growth environments and ontogenetic shifts in hydraulic and anatomical parameters. Across growth sites, ontogenetic stages and leaf orders, g_s was tightly correlated with $K_{\text{shoot-L}}$ and K_{leaf}, balancing hydraulic supply with demand for the earliest stages of seedling establishment.

Introduction

Successful seedling establishment is a major determinant of natural regeneration for many forest trees, shaping the environmental distribution of plant species and the composition of natural communities (Grubb, 1977; Clark *et al.*, 1998). Although successful seedling establishment strongly depends on sufficient photosynthetic carbon supply (Brodribb *et al.*, 2010; Johnson *et al.*, 2011; Augustine & Reinhardt, 2019), little is known of the potential constraints of hydraulic design on gas exchange and productivity in seedlings even as they undergo strong ontogenetic shifts in form and physiology. In this study, '*seedling*' strictly refers to plants in their first year, though previous studies have applied the term for several year-old plants (Kerr *et al.*, 2015).

There is a rich literature on juvenile (i.e. few years old) and mature plants showing that hydraulic conductance (*K*) is a major constraint on stomatal conductance (g_s) and photosynthetic rate, such that within and across species, hydraulic supply matches demand at whole plant scale (Sperry *et al.*, 1993a,b; Brodribb & Feild, 2000; Nardini & Salleo, 2000; Hubbard *et al.*, 2001; Brodribb *et al.*, 2017). This coordination has been proposed to arise from hydraulic optimization, such that plants would remain above a threshold leaf water potential to avoid hydraulic failure, while avoiding over-investment in water transport tissues (Mencuccini, 2003; Way *et al.*, 2014; Scoffoni *et al.*, 2016; Trugman *et al.*, 2019; Wang *et al.*, 2020). Accordingly, *K* of organs and whole plants varies strongly within given species across growth conditions (e.g. Tognetti *et al.*, 1997; Johnson *et al.*, 2011; Way *et al.*, 2013; Wang *et al.*, 2016) and ontogenetic stages (e.g. Mencuccini & Grace, 1996; Mencuccini *et al.*, 1997; Fischer *et al.*, 2002). Consistent with plants developing hydraulic capacity to meet evaporative demand (Tyree & Zimmermann, 2002), across sites, *K* tends to be higher under conditions promoting higher gas exchange rates, that is, with greater irradiance, temperature and water supply (e.g. Cochard *et al.*, 2000; Lemoine *et al.*, 2001; Brodribb *et al.*, 2005; Beikircher & Mayr, 2009; Raimondo *et al.*, 2009; Way *et al.*, 2013), and lower under high CO_2 (e.g. Heath *et al.*, 1997; Domec *et al.*, 2017). Across ontogeny, larger organs may have higher *K* in absolute terms, due to a greater number of flow paths (Becker *et al.*, 1999; Mencuccini, 2003; Sack *et al.*, 2003). Yet, plant *K* normalized by leaf area tends to decline in larger trees, as fewer and/or narrower conduits are formed in more distal parts, an effect not fully compensated by widening of conduits at the plant base or by the reduction in leaf-sapwood area ratio (Mencuccini & Grace, 1996; Hubbard *et al.*, 1999; Mencuccini & Magnani, 2000; Prendin *et al.*, 2018).

Hydraulic-stomatal-photosynthetic coordination also holds at the scale of leaves, which represent a hydraulic bottleneck, with high hydraulic resistances both in the vein xylem and outside the xylem from the mesophyll to the stomata (e.g. Sack *et al.*, 2005; Sack & Holbrook, 2006; Brodribb & Holbrook, 2007; Brodribb & Jordan, 2008; Charra-Vaskou & Mayr, 2011; Scoffoni *et al.*, 2016).

However, understanding of hydraulic-stomatal coordination in seedlings has been fragmentary, especially for only few months old plants. Seedlings of different species 3 wk after the opening of the first leaf show remarkable co-variation in xylem and stomatal anatomical traits (Zhong *et al.*, 2020). Accordingly, also a strong coordination in physiological parameters might be expected. For seedlings older than 6-month-old plants data is available, most showing a close relationship between hydraulic and stomatal conductance (Table 1; Maherali & DeLucia, 2000; Brodribb *et al.*, 2005). However, there have only been two previous studies combining hydraulic and stomatal conductance analyses in seedlings up to 6-months-old, with contradictory findings (Table 1): while Reinhardt *et al.* (2015) observed an about sevenfold decrease in shoot K but no changes in g_s during development from 10- to 70-d-old pine seedlings, Hernández *et al.* (2010) reported a tight correlation between root K and g_s in 3- to 4-month-old Mediterranean shrubs. The paucity of data for plants less than 6-months-old is likely due to methodological challenges in K analyses. Classical methods to determine fluxes are not easily applied, as sealing of tiny and fragile samples into tubes may compress the samples and thus affect measured flows. *In vivo* sap flow measurements are also challenging due to the difficulty of attaching sensors and their inaccuracy at low flow rates (e.g.

Table 1 Synthesis of studies analyzing hydraulic conductance (or resistance) in seedlings of woody species up to 6-months-old, indicating the paucity of data for up to 6-months-old plants.

Species	Age	Parameters analyzed	Method	Reference
Theoretical calculations				
Eucalyptus grandis	1–7 wk	$k_{s(theo)}$, $k_{l(theo)}$	Calculations based on conduit diameters	Johnson *et al.* (2011)
Pseudotsuga menziesii, Larix occidentalis, Pinus ponderosa	2–10 wk	$k_{s(theo)}$, $k_{l(theo)}$	Calculations based on conduit diameters	Miller & Johnson (2017)
Pinus ponderosa	*c.* 2–6 months	$k_{s(theo)}$	Calculations based on conduit diameters	Kerr *et al.* (2015)
Measurements				
Pinus flexilis	**2–10 wk**	**$K_{aboveground}$**	**EFM**	Reinhardt *et al.* (2015)
Larix occidentalis, Pseudotsuga menziesii, Pinus ponderosa	≤ 6 wk	k_s	Flowmeter	Miller *et al.* (2020)
Mediterranean shrubs	**3–4 months**	**K_{root}**	**HPFM**	**Hernández *et al.* (2010)***
Tropical tree species	3–6 months	K_{root}	HPFM	Tyree *et al.* (1995)
Neotropical tree species	4–16 months	K_{root}, K_{shoot}	HPFM	Tyree *et al.* (1998)
Populus tremuloides	5 months	K_{plant}, $K_{aboveground}$, R_{root}, R_{stem}, R_{leaf}	HPFM	Way *et al.* (2013)
Pinus sp.	*c.* 5 months	k_s	Xylem embolism meter	Creese *et al.* (2011)
Pistacia lentiscus	*c.* 5 months	K_{root}	Gravimetrically recorded water flow	Trubat *et al.* (2012)
Prosopis chilensis, Prosopis flexuosa	6 months	k_s	Gravimetrically recorded water flow	Lauenstein *et al.* (2013)
Pinus ponderosa	***c.* 6 months**	**k_s, k_l, K_{root}**	**HPFM**	Kerr *et al.* (2015)*
Pinus canariensis	**6 months**	**K_{root}**	**HPFM**	**Luis *et al.* (2010)***
Tropical tree and shrub species	6–12 months	k_l	Gravimetrically recorded water flow	Lopez *et al.* (2005)
Pistacia lentiscus	6 months	K_{root}	Gravimetrically recorded water flow	Trubat *et al.* (2006)
Betula occidentalis	**6–8 months**	**k_s, k_l**	**Pressure-flux technique**	Saliendra *et al.* (1995)*
Acer saccharinum	6–12 months	R_{plant}, R_{root}, R_{stem}, R_{leaf}	HPFM, EFM	Tsuda & Tyree (1997)

Lines in bold indicate studies including stomatal conductance analysis, asterisks highlight studies reporting tight hydraulic-stomatal coordination. Please note that from 6 months on this list is not exhaustive. (Theoretical) xylem specific conductivity ($k_{s(theo)}$) and (theoretical) leaf-specific conductivity ($k_{l(theo)}$) of stems, hydraulic conductance (K) or resistance (R) of whole plants (K/R_{plant}), aboveground organs ($K/R_{aboveground}$), shoots (K/R_{shoot}), leaves (K/R_{leaf}) and roots (K/R_{root}).

Groot & King, 1992). In this context, the evaporative flux method (EFM), often used to measure hydraulic conductance of leaves (K_{leaf}; e.g. Sack *et al.*, 2002b; Brodribb *et al.*, 2007; Scoffoni *et al.*, 2012), or whole plants (K_{plant}; Tsuda & Tyree, 1997; Corcuera *et al.*, 2012; Ounapuu & Sellin, 2013) is a promising approach for small seedlings (also see Reinhardt *et al.*, 2015).

It is critical to establish whether hydraulic-stomatal coordination extends to seedlings of the youngest ages. Seedlings represent a demographic bottleneck for natural regeneration, and are exceptional in many aspects of their structure and function. Several studies point to extensive mortality rates in the first year, with peaks shortly after germination and/or during summer drought (Johnson & Smith, 2005; Johnson *et al.*, 2011; Reinhardt *et al.*, 2015; Augustine & Reinhardt, 2019; Garcia de Jalon *et al.*, 2020; Miller *et al.*, 2020), due at least in part to limited photosynthetic carbon gain and drought stress (e.g. Germino *et al.*, 2002; Fenner & Thompson, 2005; Reinhardt *et al.*, 2015; Brodersen *et al.*, 2019). During their first year, plants undergo strong anatomical and morphological modifications with direct and indirect impacts on physiology. In conifer seedlings for instance, Miller & Johnson (2017), Miller *et al.* (2020) and Reinhardt *et al.* (2015) reported tremendous changes in various physiological and anatomical parameters linked to carbon and water relations over the first 12 wk (i.e. 3.5 months); after the sixth week, the primary xylem was replaced with secondary xylem with smaller tracheid diameters, thicker cell walls, and distinct pit anatomy, and significant increases in leaf area occurred, overall reducing hydraulic efficiency and increasing hydraulic safety. Seedlings also have contrasting biomass allocation allometries and leaf-to-xylem ratios from larger plant sizes (Sack *et al.*, 2002a), and within the seedling stage intra- and inter-specific variation has been observed as well (Miller & Johnson, 2017; Augustine & Reinhardt, 2019). Overall, seedlings represent an exceptional life stage of trees, characterized by the necessity for rapid growth of roots and leaves to compete for water and light. Whether ontogenetic adjustments in biomass allocations as well as stomatal and xylem anatomical properties occur in tight enough coordination to enable a balancing of hydraulic supply with demand has not been fully clarified for first-year seedlings and has been largely unknown for less than six-month-old plants.

The aim of our study was to test for hydraulic-stomatal coordination in multiple contexts during the first growing season. Thereby, we focused on *Acer pseudoplatanus*, an ecologically and silviculturally important tree species in Europe. We analyzed intra-specific variation in hydraulic and stomatal conductances and related anatomical traits in *A. pseudoplatanus* seedlings emerging at a wide range of growth conditions and in different ontogenetic stages. Briefly, we compared six-wk-old seedlings emerging at a subalpine forest belt site with plants growing *c.* 600 m below in a grove on a dry gravelly lime soil and in the understory of a dense, mixed forest. Further, seeds from the same seed pool as treeline plants were grown under controlled glasshouse conditions. On those plants, measurements were repeated at different developmental stages (Table 2). Our overarching hypothesis was that across seedling ontogeny and diverse growth conditions, stomatal conductance would be coordinated with shoot hydraulic conductance and leaf hydraulic conductance, as expected from the required matching of hydraulic supply and demand. Specifically, based on studies of older plants, we expected that hydraulic conductance of shoots

Table 2 Soil type, vegetation and general environmental conditions of the contrasting study sites in which seedlings grew.

Growing site	Code	Elevation (m asl)	Soil type	Tree population	Site conditions experienced by seedlings in the summer	Ontogenetic stages analyzed
Hinterhornalm 47°20′N, 11°33′E	Treeline	1522	Scarcely	developed rendzina	Scattered mature *Acer pseudoplatanus* trees at the Alpine treeline; south-exposed	MODERATELY warm and dry, half-shady
Cotyledons and one leaf pair						
Gnadenwald 47°19′N, 11°35′E	Valley	879	Gravelly lime soil	Grove of mature *A. pseudoplatanus* trees in the Inntal valley; south-exposed	Warm, dry, half-shady	Cotyledons and one leaf pair
Innsbruck 47°15′N, 11°24′E	Forest	574	Para-brown earth	Dense, mixed forest of mature *A. pseudoplatanus, Fagus sylvatica, Picea abies* trees in the Inntal valley; north exposed	Warm, humid, shady	Cotyledons and one leaf pair
Innsbruck Botanical Garden[a] 47°16′N, 11°22′E	Glasshouse-grown treeline	600	Soil mixture for alpine plants	Single plants grown in pots and daily watered	Warm, humid, sunny	Cotyledons and one, two, four and six to seven leaf pairs

[a] Please note that the seed material originates from the same seed pool as on Hinterhornalm.

and stomatal conductance would be higher for seedlings at the Alpine treeline than at low elevation, due to their adaptation and acclimation to shorter warm periods diurnally, and shorter growing seasons in which to accomplish their growth (Körner, 2012; Table 2). Further we expected that increases in xylem area and shoot hydraulic conductance would be insufficient to overcome rapid leaf growth and that, in consequence, shoot hydraulic conductance normalized by leaf area would decrease across ontogenetic stages. Finally, we expected that, due to greater access to irradiance, leaf hydraulic conductance and stomatal conductance would increase in later-produced seedling leaves. Overall, these trends would result in hydraulic-stomatal coordination and tight correlations between hydraulic and anatomical parameters in leaves and shoots. Our findings provide new resolution of the hydraulic control of gas exchange in the first months of a tree's life, on which their survival and future distribution strongly depends.

Materials and Methods

Plant material, study sites and harvesting

Measurements were made on 6 to 18-wk-old seedlings of *A. pseudoplatanus* L. (common name 'sycamore' in Europe or 'sycamore maple' in the United States). In spring 2014, newly emerged seedlings with cotyledons and one true leaf pair were collected at natural habitats varying in environmental conditions due to elevation, soil type, forest composition, and light availability in the understory (Table 2), including sites at the Alpine treeline (henceforth 'field-grown treeline'), 600 m below in a grove (henceforth 'valley') and in a dense forest (henceforth 'forest'). Additionally, seeds were harvested in autumn 2013 from five mature trees at the treeline, germinated in a glasshouse in the Innsbruck Botanical Garden, Austria, and grown in pots on a mixture of soil designed for alpine plants (leaf mold : ground soil : coconut fiber : sand : horticultural lava : rock meal; 5 : 2 : 2 : 2 : 1 : 0.15) and watered daily (henceforth, 'glasshouse-grown treeline').

Before harvesting for hydraulic measurements, plants were covered with dark nylon bags to stop transpiration for 30 min, after which individuals were carefully dug out with the whole radicle, put into a beaker with water, covered with a dark nylon bag containing wet paper towels and transported to the laboratory. There, plants were kept in a dark temperature chamber (5°C) and measured within 2 d.

Experimental design

We tested variation in hydraulic and anatomical parameters of *A. pseudoplatanus* seedlings among growth sites, during ontogeny, and across early- to late-developed leaf orders (see Supporting Information Fig. S1B). For testing the variation among growth sites (see Table 2), we measured seedlings with cotyledons and one leaf pair (LP1; *c.* 6-wk-old). For testing ontogenetic shifts in hydraulic and anatomical parameters, further measurements were carried out on glasshouse-grown treeline seedlings with two (LP2; *c.* 9-wk-old), four (LP4; *c.* 15-wk-old) and six to seven (LP6/7; *c.* 18-wk-old) leaf pairs. In the latter two stages, cotyledons were either already shed or yellowish-brown. Plants were measured for their ontogenetic stage when the following leaf pair started to develop (see Fig. S1). At that time, last developed leaves, defining the ontogenetic stage, were not yet fully expanded (*c.* 50% expanded compared to leaves at the end of the growing season). This approach allowed us to clearly define stages, though it may complicate the comparison of our data with data on fully developed leaves (see the Discussion section). As measurements were destructive, different seedlings from the same growth cohort were used when they had reached those growth stages.

For testing variation among leaf orders, we measured leaf pairs on five glasshouse-grown treeline seedlings at the end of the growing season. Studied plants had six fully developed leaf pairs which were numbered in the order of development (i.e. acropetally) with leaf order one being the basal leaf pair (after the cotyledons) and leaf order six the apical pair, most recently developed.

Measurements of hydraulic conductance

The *K* value of seedling shoots and leaves was measured using the EFM (Sack *et al.*, 2002b). The EFM operates based on the Ohm's law analogy (van den Honert, 1948; Tyree & Ewers, 1991; Tyree, 1997), which assumes steady state of water flux, enabling determination of *K* as a function of flux (*F*) and the water potential difference ($\Delta\Psi$) across the plant or plant section:

$$K = F/\Delta\Psi \qquad \text{Eqn 1}$$

Absolute shoot hydraulic conductance (K_{shoot}; in mmol s^{-1} MPa^{-1}), leaf area-specific shoot hydraulic conductance ($K_{\mathrm{shoot\text{-}L}}$; in mmol m^{-2} s^{-1} MPa^{-1}) and xylem area-specific shoot hydraulic conductance ($K_{\mathrm{shoot\_Axyl}}$; in mmol m^{-2} s^{-1} MPa^{-1}) were measured on three to 10 individual seedlings for each site and ontogenetic stage. Leaf hydraulic conductance (K_{leaf}, in mmol m^{-2} s^{-1} MPa^{-1}) of different leaf orders was analyzed on each leaf of five individual plants (i.e. 10 leaves per order). Seedlings or leaves were cut under water at the hypocotyl and petiole base, respectively, and placed in a micro test tube (Eppendorf$^{®}$) filled with flow solution. The flow solution was distilled water containing calcium chloride ($CaCl_2$, 1 mmol) and potassium chloride (KCl, 10 mmol) to avoid bacterial growth and to standardize for potential ionic effects on *K* as reported for several *Acer* species (Nardini *et al.*, 2012a), that was filtered (using a 0.22 μm filter; Sartorius, Göttingen, Germany), and degassed. The tube was tightly sealed with Parafilm$^{®}$ 'M' (Pechiney Plastic Packaging, Menasha, WI, USA) around the hypocotyl/petiole to prevent water loss over the open water surface. To avoid decreasing air pressure in the tube during transpiration, the Parafilm was perforated twice with a thin needle (0.5 mm diameter). Tests showed that evaporative water loss from the vial through the film accounted for only *c.* 0.7% of total water loss, and was thus negligible relative to transpiration, even for the LP1 plants with the lowest transpiration rates (see Fig. S1A for experimental setup and respective values).

Before measurements, the shoots or leaves were placed under a light source (halogen spot light, ST 200, IP 44, Brennenstuhl, Tübingen, Germany) providing *c.* 1500 µmol m^{-2} s^{-1} of photosynthetically active radiation (measured via PAR quantum sensor; Skye Instruments Ltd, Llandrindod Wells, UK) at leaf level for 20 min, to induce stomatal opening. To avoid strong temperature increases at the leaf surface due to the light source, a heat reflecting glass plate was positioned between the sample and light source, and a fan was used to remove the heat and to facilitate transpiration, such that the temperature was kept at *c.* 24°C (as continuously monitored with a thermo-hygrometer; RS Components Handelsges GmbH, Gmünd, Austria). Before and after measurements, g_s of uppermost leaves and cotyledons, respectively was measured with a steady-state leaf porometer (SC-1; Decagon Devices, Pullman, WA, USA) to ensure transpiration. When g_s values were stable in the range of previously measured maximum values of similar seedlings under full sun, the mass of the micro test tube and sample was taken eight to 10 times at 1 min intervals with an analytical balance (Sartorius BP61S, 0.1 mg precision, Sartorius AG, Göttingen, Germany), with the tubes replaced under the light source between weighing. The rate of mass loss (representing water flow) showed < 10% variation among measurements with no decrease during the measurement time, indicating approximately steady-state flow (Sack *et al.*, 2002). After the last measurement, the sample was removed from the micro test tube and equilibrated in a nylon bag with wet paper towels for 20 min. Then, water potential (Ψ) was measured with a pressure chamber (Model 1000 Pressure Chamber; PMS Instrument Co., Corvallis, OR, USA), and leaf area (LA) determined. The difference in mass F (mmol s^{-1}) was averaged across time intervals, and divided by $-\Psi$ to obtain K_{shoot} and further divided by total LA ($\mathrm{LA_{tot}}$) to obtain $K_{\text{shoot-L}}$ and leaf area of the respective leaf to obtain K_{leaf}. Values were then averaged for each site and ontogenetic stage. To compare leaf orders, values were first averaged for each individual and then averaged for each leaf order. To obtain $K_{\mathrm{shoot\_Axyl}}$, mean K_{shoot} was divided by the mean stem xylem area (see section 'Xylem anatomy and Huber value') of the respective growth site. Standard errors for $K_{\mathrm{shoot\_Axyl}}$ were estimated using a propagation of error formula based on the standard errors of K_{shoot} and mean xylem area (A_{xyl}) (Dunlap & Silver, 1986).

Leaf and stomatal characteristics

After K measurements, from each seedling one cotyledon and one true leaf per leaf pair were oven dried at 80°C for 48 h to obtain dry mass (DM) and calculate specific leaf area (SLA; in cm^2 g^{-1}). The other cotyledon and leaf were used for stomatal analyses. A coat of clear nail varnish was applied abaxially on intercostal areas, and when dry, peeled off with adhesive tape, placed on a microscope slide and analyzed with a light microscope (Olympus BX 41; System Microscope, Olympus Austria, Vienna, Austria) interfaced with a digital microscope camera (ProgRes CT3; Jenoptik, Jena, Germany), for determination of stomatal density (SD; in number per mm^2) and stomatal pore length (SPL; in micrometers). Observations were made at a magnification of ×10 in a field of view of 0.27 mm^2 for SD, and ×40 and 0.06 mm^2 for SPL, respectively. Measurements were made using image analysis software (IMAGEJ v.1.37; National Institutes of Health (NIH), Bethesda, MD, USA). For comparisons of seedlings across sites, SPL was analyzed on 45–75 and 155–255 stomata for each cotyledon or true leaf, respectively, and for comparisons across leaf orders, on 30–75 stomata for each leaf.

Xylem anatomy and Huber value

Hypocotyls (shoot base to onset of cotyledons) of seedlings used for K measurements were preserved in an ethanol–glycerol–water solution (1 : 1 : 1, v/v/v) for several weeks. From the central part of three to five randomly chosen hypocotyls for each site and ontogenetic stage, transverse sections were cut with a microtome (Sledge Microtome GSL 1; Schenkung Dapples, Zurich, Switzerland) and stained for lignin with phloroglucinol–hydrochloric acid. Anatomical parameters were analyzed with a light microscope (magnification ×10, field of view 0.27 mm^2) interfaced with a digital microscope camera and image analysis software (as described for stomata characteristics). From 215 to 876 individually measured xylem conduit lumen areas (A) per individual, diameters (d; in micrometers) were calculated assuming that conduits were circular and averaged to obtain mean diameter (d_{mean}), and the mean hydraulic diameter (d_{h}; in micrometers; Sperry *et al.*, 1994) as:

$$d_{\mathrm{h}} = \sum d^5 / \sum d^4 \qquad \text{Eqn 2}$$

Further, to determine cell wall reinforcement ($(t/b)^2$; Hacke *et al.*, 2001), for each hypocotyl transverse section, the double wall thickness (t) and conduit diameter (b) of the largest conduit within 9 to 12 conduit groups were determined (conduits were solitary or in groups of up to six conduits in a radial line). To avoid unbalanced statistical weighting of samples with larger numbers of analyzed conduits or stomata, values were first averaged for each individual and then averaged for each site, ontogenetic stage or leaf order (Beikircher *et al.*, 2013).

The Huber value (HV; in m^2 m^{-2}), that is, the ratio of $\mathrm{LA_{tot}}$ to sapwood xylem area, was calculated by dividing A_{xyl} by mean leaf area for each group. Furthermore, A_{xyl} was calculated for each transverse section from pith and xylem diameters (five per sample), using the formula for the area of an annulus:

$$A_{xyl} = (\pi/4) \times (d_{\mathrm{X+P}}^2 - d_{\mathrm{P}}^2) \qquad \text{Eqn 3}$$

where $d_{\mathrm{X+P}}$ is the diameter of the disk including pith and xylem and d_{P} that of pith alone. As leaf areas and xylem cross-sections were determined for different individuals in given treatments, the standard errors for HVs were estimated using a propagation of error formula based on the standard errors of A_{xyl} and $\mathrm{LA_{tot}}$ means (Dunlap & Silver, 1986).

Statistics

Differences across sites, ontogenetic stages or leaf orders were tested for all parameters except K_{shoot_Axyl} and HV with analyses of variance (ANOVA) using SPSS (v.21; SPSS, Chicago, IL, USA), after first assuring that data satisfied the Kolmogorov–Smirnov test for normality; pairwise tests were then conducted using Bonferroni or Tamhane tests, depending on their satisfying or not, respectively, the Levene's test for equal variance. As K_{shoot_Axyl} and HV were calculated for each treatment based on mean values, differences between treatments were tested with the Welch test (Rasch *et al.*, 2011). Linear regression analyses were also performed using SPSS.

Results

Variation in hydraulic and anatomical traits across natural habitats

Seedlings emerging at natural low elevation sites, that is, valley and forest, were statistically similar in their hydraulic and anatomical traits, and significantly differed from treeline seedlings in seven out of 17 analyzed parameters (Fig. 1; Table 3). Treeline seedlings had *c.* 1.3 times longer shoots than those of low elevation but similar A_{xyl}. Neither absolute (K_{shoot}) nor xylem area-specific (K_{shoot_Axyl}) shoot hydraulic conductance differed significantly among habitats. In contrast, due to the *c.* 1.4-fold lower LA_{tot} (Table 3), treeline seedlings had *c.* 40% higher leaf $K_{shoot\text{-}L}$ than low elevation seedlings (Fig. 1).

Consistent with their high $K_{shoot\text{-}L}$, treeline seedlings also had the highest values for g_s of cotyledons and first true leaves (Table 3). Across natural habitats, g_s of first true leaves was positively related with SD ($R^2 = 0.89$, $P = 0.05$), which was *c.* 1.8 times as high for treeline than low elevation seedlings. Further, in cotyledons g_s was also strongly correlated with SPL ($R^2 = 0.87$, $P = 0.05$). The SLA of the first true leaf was statistically similar across sites, at *c.* 39 $m^2\ kg^{-1}$. Xylem anatomical traits, that is, mean and hydraulic conduit diameter (d_{mean}, d_h) and cell wall reinforcement ($(t/b)^2$) were similar across habitats.

Hydraulic and anatomical adjustments under glasshouse conditions

Field- and glasshouse-grown treeline seedlings differed significantly in 10 out of 17 analyzed parameters (Fig. 1; Table 3). Despite 1.5-fold shorter shoots and 1.4-fold higher A_{xyl}, glasshouse-grown seedlings tended to have lower K_{shoot} compared to field-grown plants, which could be attributed to the 8% greater d_{mean} in latter. The significantly higher A_{xyl} and LA_{tot} of glasshouse-grown seedlings resulted in a significant reduction in $K_{shoot\text{-}L}$ and K_{shoot_Axyl} (Fig. 1b,c; Table 3). Consistent with lower $K_{shoot\text{-}L}$, g_s of both cotyledons and first true leaves were about half as high for glasshouse-grown than field-grown seedlings, which in turn was related to lower SD and SPL in former seedlings.

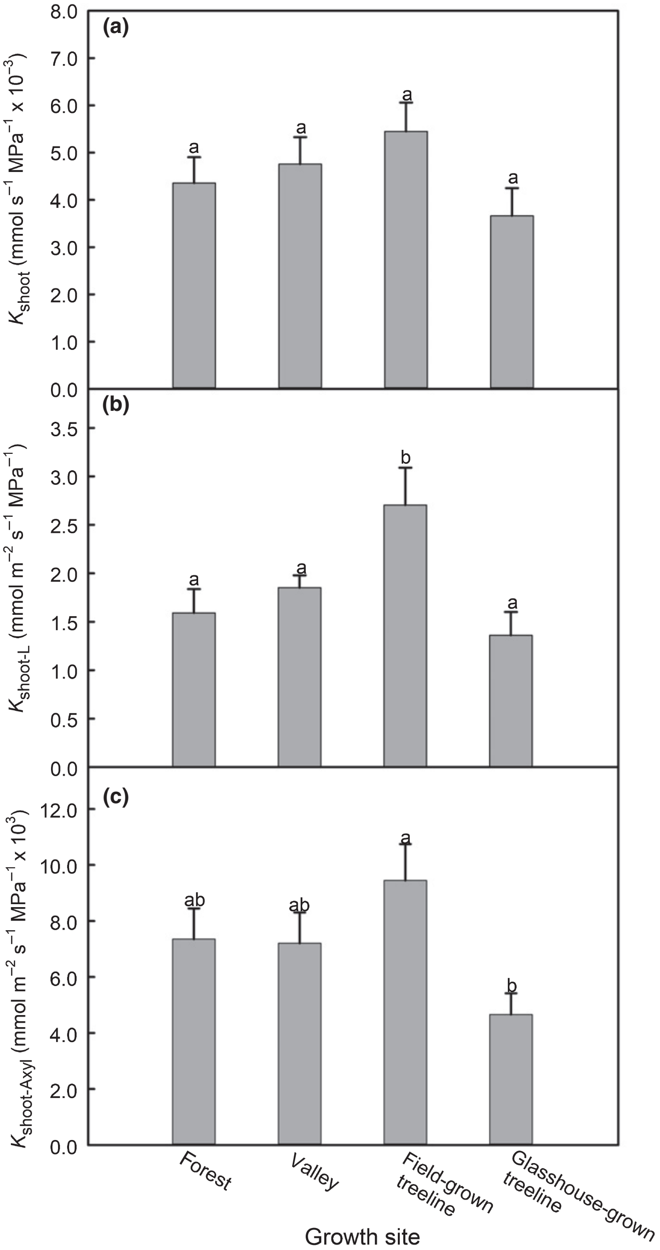

Fig. 1 (a) Absolute (K_{shoot}), (b) leaf-specific ($K_{shoot\text{-}L}$) and (c) xylem area-specific (K_{shoot_Axyl}) shoot hydraulic conductance of *Acer pseudoplatanus* seedlings with cotyledons and one true leaf pair emerging at different natural sites and a glasshouse, respectively. Treeline and glasshouse seedlings originate from the same seed pool. Significant differences ($P < 0.05$) are indicated by letters. Mean ± SE.

Ontogenetic shifts in hydraulic and anatomical parameters

Strong shifts in hydraulic conductance occurred during the transition from the first true leaf pair stage (LP1) to the largest stage studied (LP6/7), simultaneously with changes in morphology and xylem anatomy. The K_{shoot} increased four-fold in association with 10% and 40% increases in d_{mean} and d_h, respectively, and six-fold and eight-fold increases in A_{xyl} and LA_{tot}, respectively, all changes being statistically significant (Fig. 2a; Table 3). The

Table 3 Hydraulic and anatomical parameters of leaves and shoots of *Acer pseudoplatanus* seedlings with cotyledons and one true leaf pair emerging in the understory of a forest and a grove (valley) at low elevation and in the understory of mature trees at the Alpine treeline, as well as glasshouse-grown treeline plants with one (LP1), two (LP2), four (LP4) or six to seven (LP6/7) leaf pairs, respectively.

	Valley	Forest	Treeline	Glasshouse-grown treeline			
	LP1	LP1	LP1	LP1	LP2	LP4	LP6/7
g_s cotyledon (mmol m^{-2} s^{-1})	105 ± 11.7^{a}	101 ± 39.4^{a}	*135 ± 24.3^{b}*	**63 ± 2.3^{a}**	–	–	–
g_s first leaf (mmol m^{-2} s^{-1})	176 ± 9.8^{a}	133 ± 10.3^{ac}	*218 ± 24.4^{b}*	**117 ± 6.0^{c}**	–	–	–
LA_{tot} (cm^2)	27.9 ± 3.32^{a}	30.4 ± 2.14^{a}	*20.4 ± 1.17^{b}*	**$33.4 \pm 2.80^{a\alpha}$**	$65.1 \pm 0.64^{\alpha}$	$201 \pm 24.4^{\beta}$	$280 \pm 42.3^{\gamma}$
SLA first leaf (m^2 kg^{-1})	39.9 ± 1.46^{a}	36.5 ± 0.98^{a}	40.4 ± 1.58^{a}	39.0 ± 1.35^{a}	–	–	–
SD cotyledon (no mm^{-2})	90.4 ± 11.3^{a}	84.8 ± 5.16^{a}	104 ± 13.8^{a}	85.4 ± 5.54^{a}	–	–	–
SD first leaf (no mm^{-2})	249 ± 20.5^{b}	207 ± 11.8^{ab}	*419 ± 42.3^{c}*	**183 ± 10.4^{a}**	–	–	–
SPL cotyledon (μm)	18.3 ± 2.51^{a}	17.2 ± 0.43^{a}	*19.6 ± 1.65^{b}*	**14.1 ± 1.03^{c}**	–	–	–
SPL first leaf (μm)	12.3 ± 0.53^{a}	12.9 ± 0.29^{ab}	13.2 ± 0.48^{b}	12.7 ± 0.58^{ab}	–	–	–
Shoot length (mm)	46.0 ± 2.80^{a}	48.3 ± 2.32^{a}	*60.9 ± 3.20^{b}*	**$41.0 \pm 2.77^{a\alpha}$**	$66.7 \pm 10.0^{\beta}$	$98.5 \pm 12.1^{\gamma}$	$158 \pm 7.3^{\delta}$
A_{xyl} (mm^2)	0.66 ± 0.06^{ab}	0.59 ± 0.05^{a}	0.58 ± 0.05^{a}	**$0.79 \pm 0.03^{b\alpha}$**	$1.13 \pm 0.07^{\beta}$	$3.50 \pm 0.23^{\gamma}$	$4.98 \pm 0.24^{\delta}$
HV (m^2 $m^{-2} \times 10^{-4}$)	2.36 ± 0.36^{ab}	1.95 ± 0.21^{b}	2.83 ± 0.28^{a}	$2.35 \pm 0.21^{ab\alpha}$	$1.74 \pm 0.11^{\beta}$	$1.74 \pm 0.10^{\beta}$	$1.78 \pm 0.12^{\beta}$
d_{mean} (μm)	11.8 ± 0.20^{ab}	12.1 ± 0.23^{ab}	12.5 ± 0.19^{a}	**$11.5 \pm 0.17^{b\alpha}$**	$12.4 \pm 0.15^{\alpha\beta}$	$12.9 \pm 0.61^{\beta}$	$13.0 \pm 0.14^{\beta}$
d_h (μm)	15.7 ± 0.37^{a}	16.5 ± 0.68^{a}	15.7 ± 0.49^{a}	$15.5 \pm 0.26^{a\alpha}$	$17.6 \pm 0.72^{\alpha}$	$21.7 \pm 1.50^{\beta}$	$25.5 \pm 0.26^{\gamma}$
$(t/b)^2$	0.024 ± 0.006^{a}	0.026 ± 0.004^{a}	0.031 ± 0.001^{a}	$0.020 \pm 0.003^{a\alpha}$	$0.020 \pm 0.002^{\alpha}$	$0.021 \pm 0.001^{\alpha}$	$0.028 \pm 0.006^{\beta}$

Mean ± SE. Site-specific significant differences ($P < 0.05$) among seedlings with cotyledons and one true leaf pair are indicated by Latin letters (a, b, c), significant differences related to the ontogenetic stage of seedling grown in the glasshouse by Greek letters (α, β, γ). Bold and italic numbers in the columns 'glasshouse-grown treeline LP1' and 'treeline LP1', respectively indicate statistically significant differences between field- and glasshouse-grown treeline seedlings, and between high and low elevation (forest, valley) seedlings, respectively. Seeds of glasshouse plants originated from the same seed pool as treeline seedlings. Leaf pair (LP), stomatal conductance (g_s), total leaf area (LA_{tot}), specific leaf area (SLA), stomatal density (SD), stomatal pore length (SPL), xylem area (A_{xyl}), Huber value (HV), mean and hydraulic conduit diameter (d_{mean}, d_h) and cell wall reinforcement ($(t/b)^2$).

increases in both A_{xyl} and LA_{tot} resulted in approximately constant HV from LP2 to LP6/7. In contrast, $(t/b)^2$ was constant from LP1 to LP4 but significantly higher in LP6/7.

However, despite the strong increases in K_{shoot} with ontogeny, these were insufficient to compensate for the increase in LA_{tot} and about four times longer pathlength in the shoot. Consequently, $K_{shoot\text{-}L}$ decreased significantly by half from LP1 to the later stages. A similar, though not significant, trend was observed for K_{shoot_Axyl} (Fig. 2b,c).

The influence of leaf order on hydraulic and anatomical parameters

Leaf hydraulics and anatomy shifted strongly with leaf order. In plants with six leaf pairs, K_{leaf} and g_s increased significantly from the first to the fifth and fourth leaf pair, respectively, followed by a trend to decrease towards the sixth leaf pair (Fig. 3; Table 4). LA tended to increase from the first to the fourth leaf pair and then decreased significantly to the sixth leaf pair, whereas SLA was approximately constant for all leaf pairs. Further, SD significantly increased from first to sixth order leaves, whereas SPL decreased.

Hydraulic-stomatal coordination in *Acer pseudoplatanus* seedlings

With a strong generality across multiple environmental and developmental contexts, stomatal conductance was coordinated with leaf and shoot hydraulic conductance for *A. pseudoplatanus* seedlings. Across growth sites, g_s was tightly correlated with $K_{shoot\text{-}L}$ (Fig. 4a; $P = 0.01$). Considering ontogenetic stages, g_s of the uppermost leaves was significantly correlated with $K_{shoot\text{-}L}$ in LP1 seedlings, and across seedlings of growth stages LP2 to LP6/7(Fig. 4b; $P = 0.05$). The high variation in $K_{shoot\text{-}L}$ relative to that of g_s in LP1 seedlings resulted in a significantly different slope of its stomatal-hydraulic coordination ($P = 0.05$) relative to subsequent stages. Across leaf orders, g_s was tightly correlated with K_{leaf} (Fig. 4c; $P = 0.01$).

Discussion

Acer pseudoplatanus seedlings showed tight hydraulic-stomatal coordination, arising across seedlings grown in different sites, of different ontogenetic stages, and across leaves of different developmental order along the seedling shoot. Seedling growth showed substantial coordinated plasticity and ontogenetic shifts in leaf and shoot hydraulic and anatomical traits. While many studies have shown hydraulic-stomatal coordination across species (Mencuccini, 2003; Way *et al.*, 2014; Scoffoni *et al.*, 2016), our findings establish a yet stronger generality for this fundamental pattern across the critical stages of seedling establishment.

Intra-specific variation in seedling hydraulics

Differences across growth sites can represent genetically determined ecotypic variation and/or arise from phenotypic plasticity. In the present study, we observed a 1.1- to 2.3-fold variation in

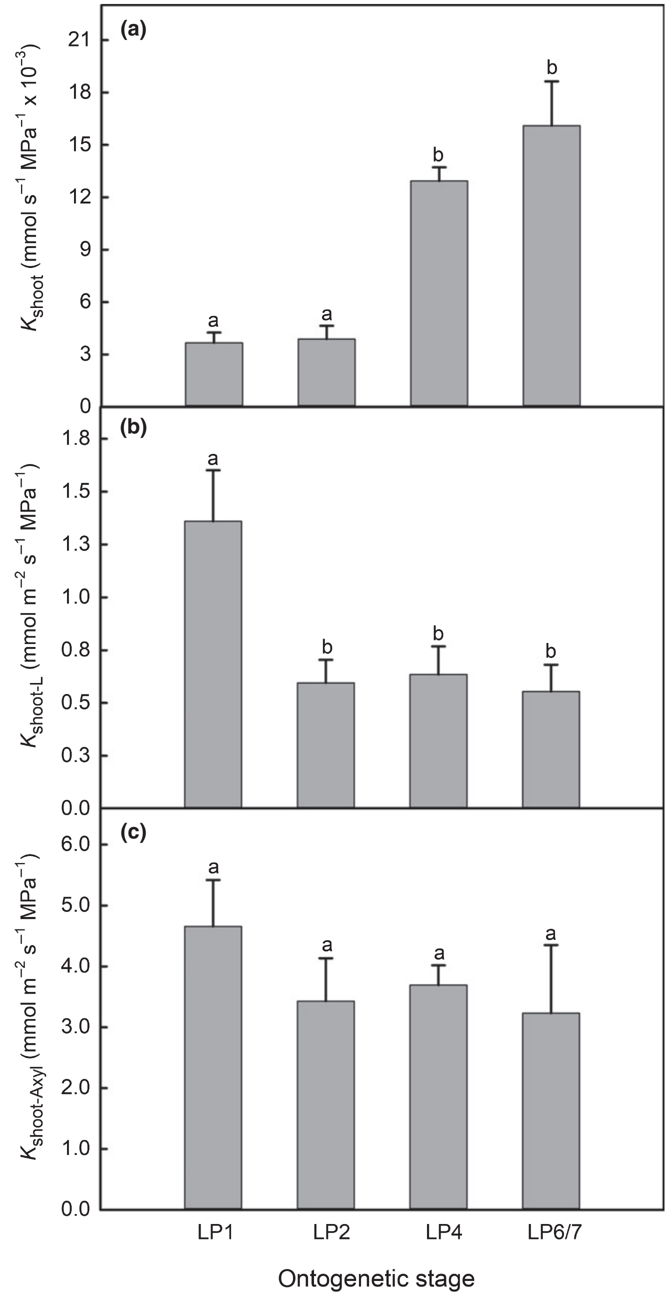

Fig. 2 Mean ± SE values for (a) absolute (K_{shoot}), (b) leaf-specific ($K_{shoot\text{-}L}$) and (c) xylem area-specific (K_{shoot_Axyl}) shoot hydraulic conductance of *Acer pseudoplatanus* seedlings with one, two, four, and six to seven leaf pairs (LP1, LP2, LP4 and LP6/7 respectively). Lowercase letters indicate statistically significant differences ($P < 0.05$).

17 hydraulic and anatomical traits in 6-wk-old *Acer* seedlings, similar to those reported in previous studies of tree seedlings, though these studies generally were based on theoretical calculations of hydraulic conductance or plants were at least 4-months-old (Maherali *et al.*, 2002; Johnson *et al.*, 2011; Lauenstein *et al.*, 2013; Way *et al.*, 2013; Kerr *et al.*, 2015). In our study, we thus importantly extended the demonstration of hydraulic adjustments in tree seedlings to actual measurements of hydraulic conductance in 6-wk-old plants.

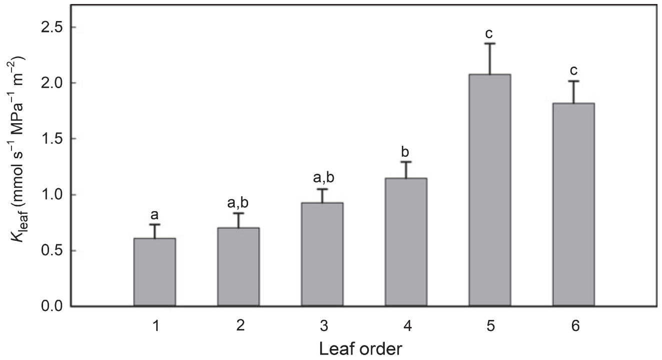

Fig. 3 Mean ± SE values for leaf hydraulic conductance (K_{leaf}) of basal (leaf order one) to apical (leaf order six) leaves of *Acer pseudoplatanus* seedlings with six leaf pairs. Lowercase letters indicate statistically significant differences ($P < 0.05$).

Field-grown treeline seedlings with one leaf pair (LP1) had significantly longer shoots (*c.* 6 cm) than seedlings at natural low elevations and glasshouse-grown seedlings (4–5 cm) in the same ontogenetic stage (Table 3). Although growth rates might be expected to decrease with altitude and especially in harsh environments, in which escaping the protective layer increases exposure to extreme temperatures and high wind speeds (Körner, 2003; Johnson *et al.*, 2011), treeline seedlings have better access to sunlight which, considering the dependence of current-year photosynthates (Augustine & Reinhardt, 2019), would be advantageous in environments with short growing seasons. Notably, despite higher shoots at similar or even lower A_{xyl}, absolute K_{shoot} of treeline plants was similar to that in low elevation and glasshouse-grown plants, indicating that the gain in path-length did not result in strong additional resistance within the integrated shoot system for these seedlings less than 10 cm tall. In contrast to K_{shoot}, the $K_{shoot\text{-}L}$ was *c.* 30–40% higher in treeline seedlings than in lowland seedlings and almost two-fold higher than in glasshouse-grown seedlings, which can be related to the lower LA_{tot} in former. As expected from the matching of hydraulic supply and demand, g_s was highest for treeline seedlings, consistent with the greater stomatal densities of their first true leaves (Table 3). Higher g_s tends to correspond to a larger number of smaller stomata (e.g. Franks *et al.*, 2009; Drake *et al.*, 2013; Sack & Buckley, 2016). Previous studies on seven temperate deciduous trees, including *A. platanoides* (Aasamaa *et al.*, 2001; Beikircher *et al.*, 2019), also suggested a role for stomatal size, indicated by SPL, and we found a correlation between SPL and g_s for cotyledons. Overall, leaf traits and shoot hydraulic conductance showed more plastic variation than stem xylem anatomy, suggesting a greater conservatism in xylem anatomy, as previously reported for pine seedlings (Augustine & Reinhardt, 2019).

The greater hydraulic and stomatal conductance in treeline seedlings, and associated variation in underlying anatomy and morphology, corresponds to acclimation and/or ecotypic adaptation to high elevation conditions. Generally, atmospheric temperatures and CO_2 partial pressure decrease with altitude while precipitation, and diurnal solar radiation and high radiation extremes increase, though local cloud cover can counteract these trends (Körner, 2003). The lower LA of treeline seedlings is in accordance with other studies showing a decline in LA with altitude (e.g. Körner, 2003). Smaller leaves have lower boundary-layer resistance and thus are better coupled with air, such that they may better avoid radiation-caused chilling damage under colder high elevation temperatures (Lusk *et al*., 2018) as well as overheating under high daytime irradiance (Harrison *et al*., 2010). Indeed, the higher SD and g_s in treeline seedlings was consistent with observed adjustment to higher irradiance (e.g. Körner, 2003), and/or to lower CO_2 partial pressure at high elevation (e.g. Kouwenberg *et al*., 2007) though, given the contemporaneously higher diffusion coefficient, latter does not negatively affect photosynthesis *per se* (Körner, 2003; Smith & Johnson, 2009). Overall, taller individuals with higher shoot conductance and smaller leaves with higher SD and g_s would be expected to improve carbon gain and thus at least partially counterbalance shorter growing seasons and shorter daily growth periods at the Alpine treeline (Premoli & Brewer, 2007; Bresson *et al*., 2009).

Interestingly, the variation between field- and glasshouse-grown treeline seedlings exceeded that found among the wild habitats (Fig. 1; Table 3), and low elevation seedlings were generally similar in their traits. In contrast to our expectations, seedlings grown in the more drought-prone valley site did not differ in measured traits from those growing at the more humid and shaded forest site (Fig. 1; Table 3). The similarity may indicate that seedlings do not have the capacity to show strong plasticity with respect to water supply, and may need to accumulate information from the environment over a longer period of time before developing changes. Given the differences observed between glasshouse- and field-grown treeline seedlings, however, we propose that growing conditions during germination and early spring growth can be impactful. Notably, forest and valley seedlings were analyzed before the canopy of mature angiosperms trees was completely leafed out, and before the soil dried, in a high rainfall spring (2014 had an 18% higher rainfall than the long-term mean; http://zamg.ac.at), reducing differences in microclimate at seedling level between these habitats. Under more differing environmental conditions in spring or also later in the season when plants outgrow the protective boundary layer (e.g. Johnson *et al*., 2011), differences between valley and forest seedlings might have been more pronounced.

Ontogenetic shifts in seedling hydraulics

We found strong ontogenetic shifts in the leaf and shoot hydraulics traits of *Acer* seedlings. As hypothesized, during the transition from seedlings with one to six/seven leaf pairs (LP1 to LP6/7), K_{shoot} increased and $K_{\text{shoot-L}}$ decreased, in coordination with anatomical and morphological changes. The value of K_{shoot} was similar at stages LP1 and LP2 (i.e. from 6 to 9 wk old), as the increases in A_{xyl} and conduit diameters compensated for the greater xylem pathlength in longer hypocotyls (Table 3). By contrast, due to the doubling of LA_{tot}, $K_{\text{shoot-L}}$ declined strongly (Fig. 2; Table 3). However, as seedlings grew from LP2 to LP6/7 (*c*. 18 wk old) the greater conduit diameters (related to conduit widening; see Olson *et al*., 2020; Soriano *et al*., 2020) and higher K_{leaf} of higher leaf orders (see next section) resulted in a sufficient increase in K_{shoot} such that $K_{\text{shoot-L}}$ remained stable despite increasing LA_{tot}. A consistent ontogenetic decoupling of xylem and leaf traits was also observed for up to 1-month-old *Eucalyptus grandis* seedlings, where the increase in theoretical xylem specific conductivity was insufficient to compensate for increased LA and consequently leaf-specific conductivity remained constant under high light and decreased under low light conditions (Johnson *et al*., 2011).

The high initial $K_{\text{shoot-L}}$ of LP1 plants indicates particularly good water supply to the first leaves that declines during ontogeny as the shoot lengthens and leaf area expands. Notably, the increase in the transpiring surface due to the development of further leaves would be partially compensated by the reduced water demand per leaf area caused by the shading of earlier-developed leaves. Interestingly, the lower $K_{\text{shoot-L}}$ in larger seedlings may coincide with increased safety of the hydraulic system, as shown by a study of Miller & Johnson (2017) for week 2–10 conifer seedlings, in which changes in pit anatomy promoted increased hydraulic safety. Indeed, the 20% increase in $(t/b)^2$ observed in this study was consistent with three-fold higher $(t/b)^2$ in 6-month-old *Acer* seedlings grown under the same conditions (Losso *et al*., 2018). These findings contrast with expectations that the youngest tree stages, being entirely dependent on current-year photosynthate, generally prioritize investments into leaf area growth and thus photosynthetic capacity over costly investments into structural traits improving drought tolerance (e.g. cell wall reinforcement, root growth; Augustine & Reinhardt, 2019), and highlights the need to determining the potentially high diversity across species and environments in the ontogeny of seedling traits generally.

Dependence of leaf hydraulic conductance on seedling leaf order

The K_{leaf} depended strongly on leaf order. Previous studies indicated that K_{leaf} can vary considerably with leaf age, light exposure or water availability (Sack & Holbrook, 2006), as well as over the course of leaf ontogeny (e.g. Aasamaa *et al*., 2005; Lo Gullo *et al*., 2005), between juvenile vs adult trees (e.g. Ishida *et al*., 2005; Sellin *et al*., 2015) and within the canopy of mature trees (e.g. Sack *et al*., 2006; Sellin, 2010). We show an analogous variation in a critical new context, that is, over the course of tree seedlings' first growing season.

As expected from the scaling of K_{leaf} with K_{plant} and the strong correlation between K_{leaf} and g_s (Sack *et al*., 2003; Sack & Holbrook, 2006), both K_{leaf} and g_s increased acropetally with leaf order (Fig. 3; Table 4). Thus, paralleling the increase of K_{shoot} from LP1 to LP6/7, during ontogeny leaves supporting successively higher K_{leaf} were developed. Increasing K_{leaf} would contribute to the seedlings's ability to meet increasing evaporative demand as distal leaves access greater levels of photosynthetic active radiation, and develop thicker leaves with greater numbers of cell layers to conduct photosynthesis (Sack *et al*., 2003; Ishida *et al*., 2005; Coste *et al*., 2009). Notably, the maximum K_{leaf} of *c*.

Table 4 Hydraulic and leaf anatomical parameters of leaves varying in order from basal to apical in *Acer pseudoplatanus* seedlings with six leaf pairs.

	Leaf order					
	1	2	3	4	5	6
g_s (mmol m^{-2} s^{-1})	134 ± 11.3^{a}	161 ± 26.0^{ab}	176 ± 28.0^{ab}	206 ± 27.3^{b}	233 ± 37.3^{b}	221 ± 12.7^{b}
LA (cm^2)	31.0 ± 4.38^{ab}	30.7 ± 4.37^{ab}	35.5 ± 2.74^{ab}	40.6 ± 2.01^{b}	23.6 ± 5.02^{ac}	21.1 ± 3.83^{ac}
SLA (m^2 kg^{-1})	30.8 ± 2.87^{a}	30.9 ± 2.68^{a}	29.7 ± 1.69^{a}	26.1 ± 0.97^{a}	26.9 ± 1.83^{a}	29.7 ± 2.04^{a}
SD (no mm^{-2})	149 ± 12.2^{a}	191 ± 10.2^{ab}	215 ± 15.7^{abc}	254 ± 40.6^{bc}	279 ± 25.0^{cd}	347 ± 37.2^{d}
SPL (µm)	11.9 ± 0.88^{ab}	12.2 ± 0.32^{b}	11.1 ± 0.29^{acd}	10.7 ± 0.56^{ac}	9.7 ± 0.58^{c}	10.2 ± 0.65^{cd}

Mean ± SE. Different letters indicate significant differences ($P < 0.05$) among different leaf orders. Stomatal conductance (g_s), leaf area (LA), specific leaf area (SLA), stomatal density (SD) and stomatal pore length (SPL).

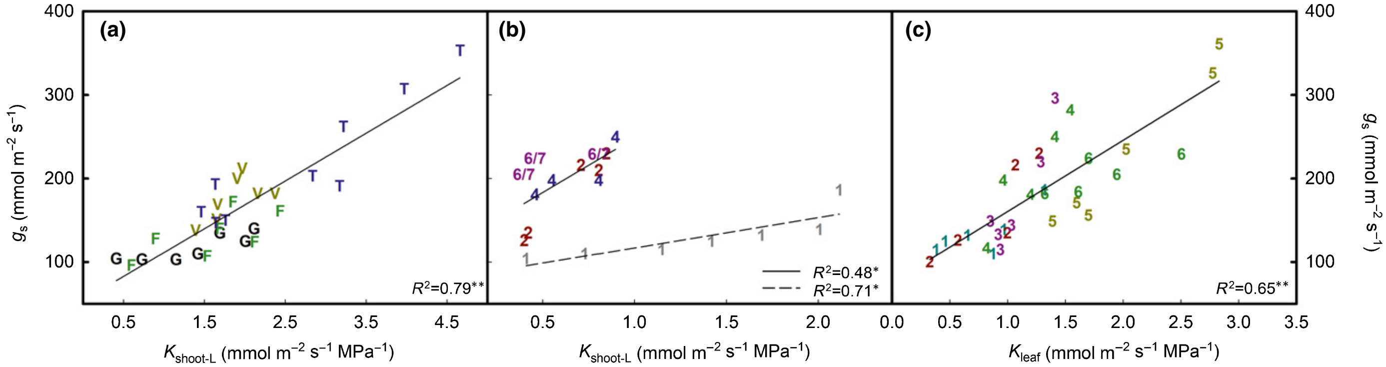

Fig. 4 Coordination of hydraulic and stomatal conductance across contexts in seedling establishment for *Acer pseudoplatanus* (a) in environmental plasticity and/or adaptation of stomatal conductance (g_s) and leaf-specific shoot hydraulic conductance ($K_{shoot\text{-}L}$), that is, for seedlings with one true leaf pair emerging at different natural sites (V = valley, F = forest, T = treeline) and glasshouse-grown treeline seedlings (G), (b) in different ontogenetic stages, that is, g_s and $K_{shoot\text{-}L}$ of glasshouse-grown seedlings with one (LP1), two (LP2), four (LP4) and six to seven (LP6/7) leaf pairs, and (c) in the shift of g_s and leaf hydraulic conductance (K_{leaf}) with leaf order (leaf order one to six, from base to apex) for seedlings with six leaf pairs. In comparing growth sites in (a), g_s measurements were made on first true leaves, while at the ontogenetic level in (b), measurements were made on the uppermost leaf. Solid lines show linear regression lines for (a) growth sites, (b) ontogenetic stages LP2 to LP6/7, and (c) leaf orders, dotted line in (b) shows linear regression for the ontogenetic stage LP1. Asterisks show statistical significance (*, $P < 0.05$; **, $P < 0.01$).

2 mmol m^{-2} s^{-1} MPa^{-1} for fifth order leaves was remarkably lower than values reported for leaves of mature *A. pseudoplatanus* trees (*c.* 13 mmol m^{-2} s^{-1} MPa^{-1}; Nardini *et al.*, 2012b), while g_s values were similar to those reported for mature trees (Lemoine *et al.*, 2001). Thus, compared with adult trees, seedlings have apparently high g_s relative to their K_{leaf}, which corresponding to their strong dependency on photosynthetic carbon gain for growth and building up carbon reserves, enables high gas exchange rates but also requires sufficient water supply and might result in large drops in water potential during the day (Brodribb *et al.*, 2010; Augustine & Reinhardt, 2019).

For the experimental seedlings, the g_s of leaves increased from the base to apex, corresponding to an increase in SD, while SPL tended to decrease. Overall, g_s was well correlated with K_{leaf} across leaf orders (Fig. 4c). Thus, acropetal increases in K_{leaf} would compensate at least partially for longer water transport paths in growing seedlings and to maintain hydraulic supply to the leaf mesophyll.

Hydraulic-stomatal coordination in seedlings in multiple contexts

A strong relationship between hydraulic supply, stomatal conductance and photosynthetic capacity has previously been reported on an evolutionary scale across diverse taxonomic groups (Brodribb & Feild, 2000; Nardini & Salleo, 2000; Brodribb & Jordan, 2008) and across different species within the woody genus *Viburnum* (Scoffoni *et al.*, 2016), as well as in the short-term after experimental treatments to suppress the hydraulic conductance of the stem (Sperry *et al.*, 1993a; Hubbard *et al.*, 2001) or of leaves (Nardini & Salleo, 2003; Brodribb & Holbrook, 2007). The discovery of the coordination of hydraulic and stomatal conductance across contexts within first year growth importantly expands the striking generality of this physiological 'rule'.

The coordination of hydraulic and stomatal conductance in seedlings was stronger for plants across contrasting growth conditions and across leaf orders than across ontogenetic stages (Fig. 4). The coordination was associated with anatomical and morphological shifts that depended on the context. First, across 6-wk-old seedlings grown under differing conditions the strong linkage of shoot hydraulic conductance and stomatal conductance (Fig. 4a) was associated with shifts in anatomical traits and morphological variation related to hydraulic and stomatal function. Second, the linkage of shoot hydraulic conductance and stomatal conductance of the uppermost leaves across developmental stages, held distinctly for the LP1 from the LP2 to LP6/7 seedlings. In part this might be related to the hydraulic peculiarities of the youngest stages, which would allow for initial growth given adequately high water supply for establishment, despite a low hydraulic supply relative to demand. In part, this difference between growth stages might have arisen due to our experimental design: g_s was measured on the uppermost leaves and thus does not reflect the median g_s of the canopy. Thus, the higher g_s relative to $K_{\text{shoot-L}}$ for older than younger seedlings might have diminished if the canopy median g_s were considered. Our focus on the uppermost leaves for g_s measurements enabled the comparison of leaves of similar developmental stages across seedlings of different ages. However, we note that as anatomical and physiological parameters can change considerably during leaf development (e.g. Cardoso *et al.*, 2018; Pantin *et al.*, 2012), this precludes the comparison of data for developing leaves in this study with those for fully developed leaves in other studies. Finally, the relationship of leaf hydraulic conductance with stomatal conductance across leaf orders (Fig. 4c) was associated with increasing SD.

Overall, we found strong variation in hydraulic and anatomical properties, due to environmental plasticity, ontogenetic trajectories, and organ age-related changes in seedlings. Across all of this variation in multiple contexts, we found coordinated shifts in hydraulic and stomatal conductance, resulting in strong coordination consistent with hydraulic optimality theory. These findings have potentially powerful applications for modeling variation in hydraulic conductance and gas exchange across establishing plants of different sizes. Just as hydraulic function is increasingly utilized for estimating tree- and forest-scale productivity and climate change responses (e.g. Anderegg, 2015; McDowell & Allen, 2015), improving the mechanistic understanding of seedling hydraulic performance is an exciting avenue for estimating the regeneration of forest species under future climatic conditions.

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Author contributions

BB and SM planned and designed the present study. BB performed hydraulic and anatomical experiments with the help of AG and AL, and analyzed the data, with guidance from LS. BB, LS and SM prepared the manuscript. AG and AL provided input on the final version of the manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aasamaa K, Niinemets Ü, Sober A. 2005. Leaf hydraulic conductance in relation to anatomical and functional traits during *Populus tremula* leaf ontogeny. *Tree Physiology* **25**: 1409–1418.

Aasamaa K, Sober A, Rahi M. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**: 765–774.

Anderegg WRL. 2015. Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. *New Phytologist* **205**: 1008–1014.

Augustine SP, Reinhardt K. 2019. Differences in morphological and physiological plasticity in two species of first-year conifer seedlings exposed to drought result in distinct survivorship patterns. *Tree Physiology* **39**: 1446–1460.

Becker P, Tyree MT, Tsuda M. 1999. Hydraulic conductances of angiosperms versus conifers: similar transport sufficiency at the whole-plant level. *Tree Physiology* **19**: 445–452.

Beikircher B, De Cesare C, Mayr S. 2013. Hydraulics of high-yield orchard trees: a case study of three *Malus domestica* cultivars. *Tree Physiology* **33**: 1296–1307.

Beikircher B, Losso A, Gemassmer M, Jansen S, Mayr S. 2019. Does fertilization explain the extraordinary hydraulic behaviour of apple trees? *Journal of Experimental Botany* **70**: 1915–1925.

Beikircher B, Mayr S. 2009. Intraspecific differences in drought tolerance and acclimation in hydraulics of *Ligustrum vulgare* and *Viburnum lantana*. *Tree Physiology* **29**: 765–775.

Bresson CC, Kowalski AS, Kremer A, Delzon S. 2009. Evidence of altitudinal increase in photosynthetic capacity: gas exchange measurements at ambient and constant CO_2 partial pressures. *Annals of Forest Science* **66**: 505–512.

Brodersen CR, Germino MJ, Johnson DM, Reinhardt K, Smith WK, Resler LM, Bader MY, Sala A, Kueppers LM, Broll G *et al.* 2019. Seedling survival at timberline is critical to conifer mountain forest elevation and extent. *Frontiers in Forests and Global Change* **2**. doi: 10.3389/ffgc.2019.00009

Brodribb TJ, Feild TS. 2000. Stem hydraulic supply is linked to leaf photosynthetic capacity: Evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment* **23**: 1381–1388.

Brodribb TJ, Feild TS, Jordan GJ. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**: 1890–1898.

Brodribb TJ, Feild TS, Sack L. 2010. Viewing leaf structure and evolution from a hydraulic perspective. *Functional Plant Biology* **37**: 488–498.

Brodribb TJ, Holbrook NM, Hill RS. 2005. Seedling growth in conifers and angiosperms: impacts of contrasting xylem structure. *Australian Journal of Botany* **53**: 749–755.

Brodribb TJ, Holbrook NM. 2007. Forced depression of leaf hydraulic conductance in situ: effects on the leaf gas exchange of forest trees. *Functional Ecology* **21**: 705–712.

Brodribb TJ, Jordan GJ. 2008. Internal coordination between hydraulics and stomatal control in leaves. *Plant, Cell & Environment* **31**: 1557–1564.

Brodribb TJ, McAdam SA, Carins Murphy MR. 2017. Xylem and stomata, coordinated through time and space. *Plant, Cell & Environment* **40**: 872–880.

Cardoso AA, Randall JM, Jordan GJ, McAdam SAM. 2018. Extended differentiation of veins and stomata is essential for the expansion of large leaves in *Rheum rhabarbarum*. *American Journal of Botany* **105**: 1967–1974.

Charra-Vaskou K, Mayr S. 2011. The hydraulic conductivity of the xylem in conifer needles (*Picea abies* and *Pinus mugo*). *Journal of Experimental Botany* **62**: 4383–4390.

Clark JS, Macklin E, Wood L. 1998. Stages and spatial scales of recruitment in Southern Appalachian forests. *Ecological Monographs* **68**: 213–235.

Cochard H, Martin R, Gross P, Bogeat-Triboulot MB. 2000. Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. *Journal of Experimental Botany* **51**: 1255–1259.

Corcuera L, Gil-Pelegrin E, Notivol E. 2012. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* **32**: 1442–1457.

Coste S, Roggy J-C, Garraud L, Heuret P, Nicolini E, Dreyer E. 2009. Does ontogeny modulate irradiance-elicited plasticity of leaf traits in saplings of rain-forest tree species? A test with *Dicorynia guianensis* and *Tachigali melinonii* (Fabaceae, Caesalpinioideae). *Annals of Forest Science* **66**: 709.

Creese C, Benscoter AM, Maherali H. 2011. Xylem function and climate adaptation in *Pinus*. *American Journal of Botany* **98**: 1437–1445.

Domec JC, Smith DD, McCulloh KA. 2017. A synthesis of the effects of atmospheric carbon dioxide enrichment on plant hydraulics: implications for whole-plant water use efficiency and resistance to drought. *Plant, Cell & Environment* **40**: 921–937.

Drake PL, Froend RH, Franks PJ. 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany* **64**: 495–505.

Dunlap WP, Silver NC. 1986. Confidence intervals and standard errors for ratios of normal variables. *Behavior Research Methods, Instruments, & Computers* **18**: 469–471.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge, UK: Cambridge University Press, 250.

Fischer DG, Kolb TE, Dewald LE. 2002. Changes in whole-tree water relations during ontogeny of *Pinus flexilis* and *Pinus ponderosa* in a high-elevation meadow. *Tree Physiology* **22**: 675–685.

Franks PJ, Drake PL, Beerling DJ. 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. *Plant, Cell & Environment* **32**: 1737–1748.

Garcia de Jalon L, Limousin JM, Richard F, Gessler A, Peter M, Hattenschwiler S, Milcu A. 2020. Microhabitat and ectomycorrhizal effects on the establishment, growth and survival of *Quercus ilex* L. seedlings under drought. *PLoS ONE* **15**: e0229807.

Germino MJ, Smith WK, Resor AC. 2002. Conifer seedling distribution and survival in an alpine-treeline ecotone. *Plant Ecology* **162**: 157–168.

Groot A, King KM. 1992. Measurements of sap flow by the heat balance method: numerical analyses and application to coniferous seedlings. *Agricultural and Forest Meteorology* **59**: 289–308.

Grubb PJ. 1977. The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biological Reviews* **52**: 107–145.

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

Harrison SP, Prentice IC, Barboni D, Kohfeld KE, Ni J, Sutra J-P. 2010. Ecophysiological and bioclimatic foundations for a global plant functional classification. *Journal of Vegetation Science* **21**: 300–317.

Heath J, Kerstiens G, Tyree MT. 1997. Stem hydraulic conductance of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur L.*) grown in elevated CO_2. *Journal of Experimental Botany* **48**: 1487–1489.

Hernández EI, Vilagrosa A, Pausas JG, Bellot J. 2010. Morphological traits and water use strategies in seedlings of Mediterranean coexisting species. *Plant Ecology* **207**: 233–244.

van den Honert TH. 1948. Water transport in plants as a catenary process. *Discussions of the Faraday Society* **3**: 146–153.

Hubbard RM, Bond BJ, Ryan MG. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiology* **19**: 165–172.

Hubbard RM, Ryan MG, Stiller V, Sperry JS. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa Pine. *Plant, Cell & Environment* **24**: 113–121.

Ishida A, Yazaki K, Hoe AL. 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, *Macaranga gigantea*. *Tree Physiology* **25**: 513–522.

Johnson DM, McCulloh KA, Reinhardt K. 2011. The earliest stages of tree growth: Development, physiology and impacts of microclimate. In: Meinzer FC, Lachenbruch B, Dawson TE, eds. *Size- and age-related changes in tree structure and function*. Dordrecht, the Netherlands: Springer, 65–87.

Johnson DM, Smith WK. 2005. Refugial forests of the Southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. *Tree Physiology* **25**: 1379–1387.

Kerr KL, Meinzer FC, McCulloh KA, Woodruff DR, Marias DE. 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiology* **35**: 535–548.

Körner C. 2003. *Alpine plant life: functional plant ecology of high mountain ecosystems*. s: Springer-Verlag, 334.

Körner C. 2012. *Alpine Treelines: functional ecology of the global high elevation tree limits*. Basel, Switzerland: Springer, 334.

Kouwenberg LLR, Kurschner WM, McElwain JC. 2007. Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy and Geochemistry* **66**: 215–241.

Lauenstein DAL, Fernandez ME, Verga AR. 2013. Drought stress tolerance of *Prosopis chilensis* and *Prosopis flexuosa* species and their hybrids. *Trees–Structure and Function* **27**: 285–296.

Lemoine D, Peltier JP, Marigo G. 2001. Comparative studies of the water relations and the hydraulic characteristics in *Fraxinus excelsior, Acer pseudoplatanus* and *A. opalus* trees under soil water contrasted conditions. *Annals of Forest Science* **58**: 723–731.

Lo Gullo MA, Nardini A, Trifiló P, Salleo S. 2005. Diurnal and seasonal variations in leaf hydraulic conductance in evergreen and deciduous trees. *Tree Physiology* **25**: 505–512.

Lopez OR, Kursar TA, Cochard H, Tyree MT. 2005. Interspecific variation in xylem vulnerability to cavitation among tropical tree and shrub species. *Tree Physiology* **25**: 1553–1562.

Losso A, Bär A, Dämon B, Dullin C, Ganthaler A, Petruzzellis F, Savi T, Tromba G, Nardini A, Mayr S *et al.* 2018. Insights from in vivo micro-CT analysis: testing the hydraulic vulnerability segmentation in *Acer pseudoplatanus* and *Fagus sylvatica* seedlings. *New Phytologist* **221**: 2196–2207.

Luis VC, Llorca M, Chirino E, Hernández EI, Vilagrosa A. 2010. Differences in morphology, gas exchange and root hydraulic conductance before planting in *Pinus canariensis* seedlings growing under different fertilization and light regimes. *Trees* **24**: 1143–1150.

Lusk CH, Clearwater MJ, Laughlin DC, Harrison SP, Prentice IC, Nordenstahl M, Smith B. 2018. Frost and leaf-size gradients in forests: global patterns and experimental evidence. *New Phytologist* **219**: 565–573.

Maherali H, DeLucia EH. 2000. Interactive effects of elevated CO_2 and temperature on water transport in ponderosa pine. *American Journal of Botany* **87**: 243–249.

Maherali H, Williams BL, Paige KN, Delucia EH. 2002. Hydraulic differentiation of Ponderose Pine populations along a climate gradient is not associated with ecotypic divergence. *Functional Ecology* **16**: 510–521.

McDowell NG, Allen CD. 2015. Darcy's law predicts widespread forest mortality under climate warming. *Nature Climate Change* **5**: 669–672.

Mencuccini M. 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**: 163–182.

Mencuccini M, Grace J. 1996. Developmental patterns of above-ground hydraulic conductance in a Scots pine (*Pinus sylvestris* L.) age sequence. *Plant, Cell & Environment* **19**: 939–948.

Mencuccini M, Grace J, Fioravanti M. 1997. Biomechanical and hydraulic determinants of tree structure in Scots pine: anatomical characteristics. *Tree Physiology* **17**: 105–113.

Mencuccini M, Magnani F. 2000. Comment on 'hydraulic limitation of tree height: a critique' by Becker, Meinzer & Wullschleger. *Functional Ecology* **14**: 135–137.

Miller ML, Johnson DM. 2017. Vascular development in very young conifer seedlings: theoretical hydraulic capacities and potential resistance to embolism. *American Journal of Botany* **104**: 979–992.

Miller ML, Roddy AB, Brodersen CR, McElrone AJ, Johnson DM. 2020. Anatomical and hydraulic responses to desiccation in emergent conifer seedlings. *American Journal of Botany* **107**: 1177–1188.

Nardini A, Dimasi F, Klepsch M, Jansen S. 2012a. Ion-mediated enhancement of xylem hydraulic conductivity in four *Acer* species: relationships with ecological and anatomical features. *Tree Physiology* **32**: 1434–1441.

Nardini A, Peda G, La Rocca N. 2012b. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist* **196**: 788–798.

Nardini A, Salleo S. 2000. Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees-Structure and Function* **15**: 14–24.

Nardini A, Salleo S. 2003. Effects of the experimental blockage of the major veins on hydraulics and gas exchange of *Prunus laurocerasus* L. leaves. *Journal of Experimental Botany* **54**: 1213–1219.

Olson ME, Anfodillo T, Gleason SM, McCulloh KA. 2020. Tip-to-base xylem conduit widening as an adaptation: causes, consequences, and empirical priorities. *New Phytologist.* **70**: 5765–5772.

Ounapuu E, Sellin A. 2013. Daily dynamics of leaf and soil-to-branch hydraulic conductance in silver birch (*Betula pendula*) measured in situ. *Plant Physiology and Biochemistry* **68**: 104–110.

Pantin F, Simonneau T, Muller B. 2012. Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist* **196**: 349–366.

Premoli AC, Brewer CA. 2007. Environmental v. genetically driven variation in ecophysiological traits of *Nothofagus pumilio* from contrasting elevations. *Australian Journal of Botany* **55**: 585–591.

Prendin AL, Mayr S, Beikircher B, von Arx G, Petit G. 2018. Xylem anatomical adjustments prioritize hydraulic efficiency over safety as Norway spruce trees grow taller. *Tree Physiology* **38**: 1088–1097.

Raimondo F, Trifiló P, Lo Gullo MA, Buffa R, Nardini A, Salleo S. 2009. Effects of reduced irradiance on hydraulic architecture and water relations of two olive clones with different growth potentials. *Environmental and Experimental Botany* **66**: 249–256.

Rasch D, Kubinger KD, Moder K. 2011. The two-sample *t* test: pre-testing its assumptions does not pay off. *Statistical Papers* **52**: 219–231.

Reinhardt K, Germino MJ, Kueppers LM, Domec JC, Mitton J. 2015. Linking carbon and water relations to drought-induced mortality in *Pinus flexilis* seedlings. *Tree Physiology* **35**: 771–782.

Sack L, Buckley TN. 2016. The developmental basis of stomatal density and flux. *Plant Physiology* **171**: 2358–2363.

Sack L, Cowan PD, Jaikumar N, Holbrook NM. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* **26**: 1343–1356.

Sack L, Holbrook NM. 2006. Leaf hydraulics. *Annual Review of Plant Biology* **57**: 361–381.

Sack L, Marañón T, Grubb PJ. 2002a. Global allocation rules for patterns of biomass partitioning. *Science* **296**: 1923.

Sack L, Melcher PJ, Zwieniecki MA, Holbrook NM. 2002b. The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* **53**: 2177–2184.

Sack L, Melcher PJ, Liu WH, Middleton E, Pardee T. 2006. How strong is intracanopy leaf plasticity in temperate deciduous trees? *American Journal of Botany* **93**: 829–839.

Sack L, Tyree MT, Holbrook NM. 2005. Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytologist* **167**: 403–413.

Saliendra NZ, Sperry JS, Comstock JP. 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis. Planta* **196**: 357–366.

Scoffoni C, Chatelet DS, Pasquet-Kok J, Rawls M, Donoghue MJ, Edwards EJ, Sack L. 2016. Hydraulic basis for the evolution of photosynthetic productivity. *Nature Plants* **2**: 16072.

Scoffoni C, McKown AD, Rawls M, Sack L. 2012. Dynamics of leaf hydraulic conductance with water status: quantification and analysis of species differences under steady state. *Journal of Experimental Botany* **63**: 643–658.

Sellin ALK. 2010. Variation of transpiration within a canopy of silver birch: effect of canopy position and daily versus nightly water loss. *Ecohydrology* **3**: 467–477.

Sellin A, Rosenvald K, Ounapuu-Pikas E, Tullus A, Ostonen I, Lohmus K. 2015. Elevated air humidity affects hydraulic traits and tree size but not biomass allocation in young silver birches (*Betula pendula*). *Frontiers in Plant Science* **6**: 860.

Smith KS, Johnson DM. 2009. Biophysical effects of altitude on plant gas exchange. In: de la Barrera E, Smith KS, eds. *Perspectives in biophysical plant ecophysiology - A tribute to Park S. Nobel.* Mexico: Universidad Nacional Autonoma de Mexico, 257–282.

Soriano D, Echeverria A, Anfodillo T, Rosell JA, Olson ME. 2020. Hydraulic traits vary as the result of tip-to-base conduit widening in vascular plants. *Journal of Experimental Botany* **71**: 4232–4242.

Sperry JS, Alder NN, Eastlack SE. 1993a. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *Journal of Experimental Botany* **44**: 1075–1082.

Sperry JS, Alder NN, Eastlack SE. 1993b. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *Journal of Experimental Botany* **44**: 1075–1082.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* **75**: 1736–1752.

Tognetti R, Michelozzi M, Giovannelli A. 1997. Geographical variation in water relations, hydraulic architecture and terpene composition of Aleppo pine seedlings from Italian provenances. *Tree Physiology* **17**: 241–250.

Trugman AT, Anderegg LDL, Wolfe BT, Birami B, Ruehr NK, Detto M, Bartlett MK, Anderegg WRL. 2019. Climate and plant trait strategies determine tree carbon allocation to leaves and mediate future forest productivity. *Global Change Biology* **25**: 3395–3405.

Tsuda M, Tyree MT. 1997. Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum. Tree Physiology* **17**: 351–357.

Trubat R, Cortina J, Vilagrosa A. 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.). *Trees* **20**: 334–339.

Trubat R, Cortina J, Vilagrosa A. 2012. Root architecture and hydraulic conductance in nutrient deprived *Pistacia lentiscus* L. seedlings. *Oecologia* **170**: 899–908.

Tyree M. 1997. The cohesion-tension theory of sap ascent: current controversies. *Journal of Experimental Botany* **48**: 1753–1765.

Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345–360.

Tyree MT, Patino S, Bennink J, Alexander J. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany* **46**: 83–94.

Tyree MT, Velez V, Dalling JW. 1998. Growth dynamics of root and shoot hydraulic conductance in seedlings of five neotropical tree species: scaling to show possible adaptation to differing light regimes. *Oecologia* **114**: 293–298.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap.* Berlin: Springer Verlag, 98.

Wang Y, Sperry JS, Anderegg WRL, Venturas MD, Trugman AT. 2020. A theoretical and empirical assessment of stomatal optimization modeling. *New Phytologist* **227**: 311–325.

Wang AY, Wang M, Yang D, Song J, Zhang WW, Han SJ, Hao GY. 2016. Responses of hydraulics at the whole-plant level to simulated nitrogen deposition of different levels in *Fraxinus mandshurica. Tree Physiology* **36**: 1045–1055.

Way DA, Domec JC, Jackson RB. 2013. Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. *Plant, Cell, & Environment* **36**: 103–115.

Way DA, Katul GG, Manzoni S, Vico G. 2014. Increasing water use efficiency along the C3 to C4 evolutionary pathway: a stomatal optimization perspective. *Journal of Experimental Botany* **65**: 3683–3693.

Zhong M, Cerabolini BEL, Castro-Diez P, Puyravaud JP, Cornelissen JHC. 2020. Allometric co-variation of xylem and stomata across diverse woody seedlings. *Plant, Cell & Environment* **43**: 2301–2310.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Experimental setup for the measurement of hydraulic conductance of seedling shoots and leaves.

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