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

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Abstract

- 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_{Lshoot}), 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_{Lshoot}, 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_{Lshoot} 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 CO₂ (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 seven-fold 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 scaling 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    | $k_{l,(theo)}, k_{l,(theo)}$  | Calculations based on conduit diameters | Johnson et al. (2011)             |
| *Pseudotsuga menziesii*, *Larix occidentalis*, *Pinus ponderosa* | 2–10 wk   | $k_{l,(theo)}, k_{l,(theo)}$  | Calculations based on conduit diameters | Miller & Johnson (2017)           |
| *Pinus ponderosa*            | c. 2–6 months | $k_{l,(theo)}$  | 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  | $k_l$                         | HPFM                          | Luis et al. (2010)*              |
| *Pistacia lentiscus*         | 6 months  | $K_{root}$                    | Gravimetrically recorded water flow | Lopez et al. (2005)             |
| *Betula occidentalis*        | 6–8 months | $k_s$, $k_l$                  | Pressure-flux technique       | Trubat et al. (2006)             |
| *Acer saccharinum*           | 6–12 months | $R_{plant}, R_{root}, R_{stem}, R_{leaf}$ | HPFM, EFM                      | Saliendra et al. (1995)*         |

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 ($k_{l,(theo)}$) 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 (\(K_{\text{leaf}}\) e.g. Sack et al., 2002b; Brodribb et al., 2007; Scoffoni et al., 2012), or whole plants (\(K_{\text{plant}}\); 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; García 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

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

| Growing site                  | Code    | Elevation (m a.s.l) | Soil type            | Tree population                                      | Site conditions experienced by seedlings in the summer | Ontogenetic stages analyzed                     |
|-------------------------------|---------|---------------------|----------------------|-----------------------------------------------------|-------------------------------------------------------|-----------------------------------------------|
| Hinterhornalm                 | 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  |         |                     |                      |                                                     |                                                       |                                               |
| Gnadental                    | Valley  | 879                 | Gravelly lime soil   | Grove of mature *A. pseudoplatanus* trees in the Innthal valley; south-exposed | Warm, dry, half-shady                                   | Cotyledons and one leaf pair                    |
| Innsbruck                    | Forest  | 574                 | Parabrown earth soil mixture for alpine plants | Dense, mixed forest of mature *A. pseudoplatanus, Fagus sylvatica, Picea abies* trees in the Innthal valley; north exposed | Warm, humid, sunny                                   | Cotyledons and one, two, four and six to seven leaf pairs |
| Botanical Garden¹            | Glasshouse-grown treeline | 600                  |                      | Single plants grown in pots and daily watered        |                                                       |                                               |

¹Please 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 (ΔΨ) across the plant or plant section:

\[ K = \frac{F}{\Delta \Psi} \]  

Eqn 1

Absolute shoot hydraulic conductance (Kshoot; in mmol s\(^{-1}\) MPa\(^{-1}\)), leaf area-specific shoot hydraulic conductance (K\(_{\text{shoot-L}}\); in mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\)) and xylem area-specific shoot hydraulic conductance (K\(_{\text{shoot-Axyl}}\); 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 (Kleaf; 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, 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 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 weighings. 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 ($\Psi$) 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 ($\Delta$) between two measures was converted to dry mass (DM) and calculate specific leaf area (SLA; in cm$^2$ g$^{-1}$). leaf area 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 30–70 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; Schencku 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 ($d_{\text{mean}}$), and the mean hydraulic diameter ($d_h$; in micrometers; Sperry et al., 1994) as:

$$d_h = \frac{\sum d^5}{\sum d^4} \quad \text{Eqn 2}$$

Further, to determine cell wall reinforcement ($(d b)^2$; 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 LA 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:

$$A_{xyl} = \left(\frac{\pi}{4}\right) \times (d_{xyl}^2 - d_p^2) \quad \text{Eqn 3}$$

where $d_{xyl}$ 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 LA means (Dunlap & Silver, 1986).
Statistics

Differences across sites, ontogenetic stages or leaf orders were tested for all parameters except $K_{\text{shoot\_Axyl}}$ 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 $K_{\text{shoot\_Axyl}}$ 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 $A_{\text{xyt}}$. Neither absolute ($K_{\text{shout}}$) nor xylem area-specific ($K_{\text{shout\_Axyl}}$) shoot hydraulic conductance differed significantly among habitats. In contrast, due to the c. 1.4-fold lower $L_{\text{Atot}}$ (Table 3), treeline seedlings had c. 40% higher leaf $K_{\text{shout\_L}}$ than low elevation seedlings (Fig. 1). Consistent with their high $K_{\text{shout\_L}}$, 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 ($d_{\text{mean}}$, $d_h$) 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 $A_{\text{xyt}}$ glasshouse-grown seedlings tended to have lower $K_{\text{shout}}$ compared to field-grown plants, which could be attributed to the 8% greater $d_{\text{mean}}$ in latter. The significantly higher $A_{\text{xyt}}$ and $L_{\text{Atot}}$ of glasshouse-grown seedlings resulted in a significant reduction in $K_{\text{shout}}$ and $K_{\text{shout\_Axyl}}$ (Fig. 1b,c; Table 3). Consistent with lower $K_{\text{shout\_L}}$, $g_s$ 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.

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 $K_{\text{shout}}$ increased four-fold in association with 10% and 40% increases in $d_{\text{mean}}$ and $d_h$, respectively, and six-fold and eight-fold increases in $A_{\text{xyt}}$ and $L_{\text{Atot}}$, respectively, all changes being statistically significant (Fig. 2a; Table 3).
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 LP1 | Forest LP1 | Treeline LP1 | Glasshouse-grown treeline LP1 | Glasshouse-grown treeline LP2 | Glasshouse-grown treeline LP4 | Glasshouse-grown treeline LP6/7 |
|----------------|------------|------------|--------------|-------------------------------|-----------------------------|-----------------------------|-------------------------------|
| $g_s$ (mmol m$^{-2}$ s$^{-1}$) | 105 $\pm$ 11.7$^a$ | 101 $\pm$ 39.4$^a$ | 135 $\pm$ 24.3$^b$ | 63 $\pm$ 2.3$^c$ | – | – | – |
| $g_s$ (mmol m$^{-2}$ s$^{-1}$) | 176 $\pm$ 9.8$^a$ | 133 $\pm$ 10.3$^{ac}$ | 218 $\pm$ 24.4$^b$ | 117 $\pm$ 6.0$^c$ | – | – | – |
| $A_{leaf}$ (cm$^2$) | 27.9 $\pm$ 3.3$^a$ | 30.4 $\pm$ 2.14$^{ac}$ | 20.4 $\pm$ 1.77$^b$ | 33.4 $\pm$ 2.80$^{aou}$ | 66.1 $\pm$ 0.64$^a$ | 201 $\pm$ 24.4$^b$ | 280 $\pm$ 42.3$^c$ |
| SLA first leaf (m$^2$ kg$^{-1}$) | 39.9 $\pm$ 1.4$^a$ | 36.5 $\pm$ 0.98$^b$ | 40.4 $\pm$ 1.58$^c$ | 39.0 $\pm$ 1.35$^a$ | – | – | – |
| SD cotyledon (mm no-2) | 90.4 $\pm$ 11.3$^a$ | 84.8 $\pm$ 5.16$^b$ | 104 $\pm$ 13.8$^c$ | 85.4 $\pm$ 5.54$^a$ | – | – | – |
| SD first leaf (mm no-2) | 249 $\pm$ 20.5$^a$ | 207 $\pm$ 11.8$^{ab}$ | 419 $\pm$ 42.3$^c$ | 183 $\pm$ 10.4$^a$ | – | – | – |
| SPL cotyledon (µm) | 18.3 $\pm$ 2.51$^a$ | 17.2 $\pm$ 0.43$^b$ | 19.6 $\pm$ 1.65$^b$ | 14.1 $\pm$ 1.03$^c$ | – | – | – |
| SPL first leaf (µm) | 12.3 $\pm$ 0.53$^a$ | 12.9 $\pm$ 0.29$^{ab}$ | 13.2 $\pm$ 0.48$^b$ | 12.7 $\pm$ 0.58$^{ab}$ | – | – | – |
| Shoot length (mm) | 46.0 $\pm$ 2.80$^a$ | 48.3 $\pm$ 2.32$^a$ | 60.9 $\pm$ 3.20$^b$ | 41.0 $\pm$ 2.77$^{aou}$ | 66.7 $\pm$ 10.0$^b$ | 98.5 $\pm$ 12.1$^a$ | 158 $\pm$ 7.3$^c$ |
| $A_{leaf}$ (mm$^2$) | 0.66 $\pm$ 0.06$^{ab}$ | 0.59 $\pm$ 0.05$^a$ | 0.58 $\pm$ 0.05$^b$ | 0.79 $\pm$ 0.03$^{bou}$ | 1.13 $\pm$ 0.07$^b$ | 3.50 $\pm$ 0.23$^a$ | 4.98 $\pm$ 0.24$^c$ |
| HV (m$^2$ m$^{-2}$ s$^{-1}$) | 2.36 $\pm$ 0.36$^{ab}$ | 1.95 $\pm$ 0.21$^b$ | 2.83 $\pm$ 0.28$^c$ | 2.35 $\pm$ 0.21$^{abou}$ | 1.74 $\pm$ 0.11$^b$ | 1.74 $\pm$ 0.10$^c$ | 1.78 $\pm$ 0.12$^a$ |
| $d_{mean}$ (µm) | 11.8 $\pm$ 0.20$^a$ | 12.1 $\pm$ 0.23$^b$ | 12.5 $\pm$ 0.19$^{c}$ | 11.5 $\pm$ 0.17$^{bou}$ | 12.4 $\pm$ 0.15$^{ab}$ | 12.9 $\pm$ 0.61$^b$ | 13.0 $\pm$ 0.14$^d$ |
| $d_{h}$ (µm) | 15.7 $\pm$ 0.37$^a$ | 16.5 $\pm$ 0.68$^c$ | 15.7 $\pm$ 0.49$^b$ | 15.5 $\pm$ 0.26$^{bou}$ | 17.6 $\pm$ 0.72$^c$ | 21.7 $\pm$ 1.50$^a$ | 25.5 $\pm$ 0.26$^d$ |
| $(b/b)^2$ | 0.024 $\pm$ 0.006$^a$ | 0.026 $\pm$ 0.004$^a$ | 0.031 $\pm$ 0.001$^a$ | 0.020 $\pm$ 0.003$^{aou}$ | 0.020 $\pm$ 0.002$^b$ | 0.021 $\pm$ 0.001$^c$ | 0.028 $\pm$ 0.006$^d$ |

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 ($A_{leaf}$), specific leaf area (SLA), stomatal density (SD), stomatal pore length (SPL), xylem area (Axyl). Huber value (HV), mean and hydraulic conduit diameter ($d_{mean}$, $d_h$) and cell wall reinforcement $(b/b)^2$.

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

However, despite the strong increases in $K_{leaf}$ with ontogeny, these were insufficient to compensate for the increase in $A_{leaf}$ and about four times longer pathlength in the shoot. Consequently, $K_{leaf}$ decreased significantly by half from LP1 to the later stages. A similar, though not significant, trend was observed for $K_{leaf, Axyl}$ (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 $K_{leaf}$ (Fig. 4a; $P = 0.01$). Considering ontogenetic stages, $g_s$ of the uppermost leaves was significantly correlated with $K_{leaf}$ in LP1 seedlings, and across seedlings of growth stages LP2 to LP6/7(Fig. 4b; $P = 0.05$). The high variation in $K_{leaf}$ 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...
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.

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 Axyl, absolute $K_{\text{shoot}}$ 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 $K_{\text{shoot}}$, the $K_{\text{shoot-L}}$ 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, $g_c$ was highest for treeline seedlings, consistent with the greater stomatal densities of their first true leaves (Table 3). Higher $g_c$ 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 $g_c$ 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 CO₂ 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 order (Fig. 3; Table 4). Thus, paralleling the increase of \( \frac{A_{\text{at}}}{\text{leaf}} \) with \( \frac{A_{\text{at}}}{\text{leaf}} \) 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 photosynthesize, 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 \( K_{\text{leaf}} \) depended strongly on leaf order. Previous studies indicated that \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \) with \( K_{\text{plant}} \) and the strong correlation between \( K_{\text{leaf}} \) and \( g_s \) (Sack et al., 2003; Sack & Holbrook, 2006), both \( K_{\text{leaf}} \) and \( g_s \) increased acropetally with leaf order (Fig. 3; Table 4). Thus, paralleling the increase of \( K_{\text{leaf}} \) from LP1 to LP6/7, during ontogeny leaves supporting successively higher \( K_{\text{leaf}} \) were developed. Increasing \( K_{\text{leaf}} \) would contribute to the seedlings’ 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 \( K_{\text{leaf}} \) of c.
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$^2$ g$^{-1}$) | 30.8 ± 2.87$^a$ | 30.9 ± 2.68$^a$ | 29.7 ± 1.69$^a$ | 26.1 ± 0.97$^b$ | 26.9 ± 1.83$d$ | 29.7 ± 2.04$^d$ |
| SD (mm$^{-2}$) | 149 ± 12.2$^a$ | 191 ± 10.2$^{ab}$ | 215 ± 15.7$^{abc}$ | 254 ± 40.6$^{dc}$ | 279 ± 25.0$^{cd}$ | 347 ± 37.2$^{cd}$ |
| 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 ($K_{s\text{-leaf}}$) 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 $K_{s\text{-leaf}}$, 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 ($K_{s\text{leaf}}$) 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 $K_{s\text{leaf}}$, 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 $K_{s\text{leaf}}$ across leaf orders (Fig. 4c). Thus, acropetal increases in $K_{s\text{leaf}}$ 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 & Salles, 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 & Salles, 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 $K_{\text{shoot} \cdot L}$ 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.

Acknowledgements

The authors would like to thank Birgit Daimon for technical assistance and Magdalena Held for help with anatomical analysis. The authors also thank the involved gardeners of the Botanical Garden Innsbruck for their help with cultivation and nurture of plants. This study was financed by means of the Austrian Science Fund (FWF; project T667-B16 ‘Hydraulics of juvenile trees’; project J-4300 ‘Surface tension of xylem sap’; project P29896-B22 ‘Analysis of Norway spruce rust-resistance’), and conducted in the frame of the research area ‘Alpiner Raum’ of the University of Innsbruck.

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.

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