Xylem and phloem in petioles are coordinated with leaf gas exchange in oaks with contrasting anatomical strategies depending on leaf habit

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ABSTRACT

As the single link between leaves and the rest of the plant, petioles must develop conductive tissues according to the water influx and sugar outflow of the leaf lamina. A scaling relationship between leaf area and anatomical traits of xylem and phloem is expected to improve the efficiency of these tissues. However, the different constraints compromising the functionality of both tissues (e.g., risk of cavitation) must not be disregarded. Additionally, plants present two main leaf habits (deciduous and evergreen) that may have different strategies to produce and package their petiole conduits to cope with environmental restrictions. In this study, we explore, in a diverse group of 33 oak species, the relationships between petiole anatomical traits, leaf area, stomatal conductance and photosynthesis rate. Results showed allometric scaling between anatomical structure of xylem and phloem with leaf area. We also found how photosynthesis and stomatal conductance at leaf-level are correlated with anatomical traits in the petiole. Nonetheless, the main novelty is how oaks present a different strategy depending on the leaf habit. Deciduous species tend to increase their diameters to achieve a greater leaf-specific conductivity. By contrast, evergreen oaks develop larger xylem conductive areas for a given leaf area than deciduous ones. This trade-off between safety-efficiency in petioles has never been attributed to the leaf habit of the species.

INTRODUCTION

Petioles, besides accomplishing a structural function, link the main photosynthetic organs —i.e., leaf laminae— with the rest of the plant. In this regard, they may act as a bottleneck in the soil-plant-atmosphere continuum for water transport and the translocation of photosynthates (Brocious and Hacke, 2016). Thus, transpiration has been traditionally related to transport capacity of xylem and photosynthesis rate to export capacity of phloem in petioles (Salisbury, 1913; Brocious and Hacke, 2016). However, this correlation has been questioned within angiosperms (Gleason et al., 2016). These authors reported a weak or even absent coordination between hydraulic capacity and gas exchange capacity.

Petioles must contain enough xylem vessels and sieve tubes to, respectively, supply water to the leaf lamina and export assimilates from the leaf to the rest of the plant. For this reason, a scaling relationship between leaf area and both xylem and phloem structures (area of conductive tissues and size of the conduits) in petioles is expected (Ray and Jones, 2018). The number and size of the conduits ultimately reflects the transport ability —namely, the hydraulic conductivity (K_h)— of the conductive tissues according to the Hagen-Poiseuille law (Tyree and Zimmermann, 2002; Hirose et al., 2005; Woodruff, 2014). Nonetheless, the K_h only shows how much fluid is potentially able to be moved along a pathway whereas two petioles with the same K_h can support different leaf areas. For this reason, the leaf-specific conductivity (LSC) of a petiole provides a more physiological explanation of a leaf's efficiency as LSC is the capacity to supply water (K_h) per leaf area. LSC can also be expressed as the product of the specific conductivity (K_s, i.e. K_h per conductive area ratio) and the Huber value (H_v, i.e. conductive area per leaf area ratio) (Mencuccini et al., 2019). Therefore, the same LSC can be achieved in different ways by modulating both K_s and H_v. Indeed, Mencuccini et al. (2019) found in a wide range of plant species a negative relationship between K_s and H_v in stems. What are the implications of increasing each variable?

Increasing the Huber value, that is, allocating more cross-sectional area to a conductive function, would imply a reduction in the availability of space for structural support. This could result in a possible trade-off between both kind of tissues (Zwieniecki et al., 2004). By contrast, as the specific conductivity mainly depends on the diameter of the conduits, the hydraulic capacity of the petiole is compromised by the same factors that determine its vulnerability, as in any other parts of the plant (Hacke and Sauter, 1996) In fact, it has

been suggested that petioles may act as hydraulic fuses for the plant through higher vulnerability to embolism than stems, thereby ensuring resilience to extreme drought events (Peguero-Pina et al., 2015; Alonso-Forn et al., 2021). Throughout their lifespan, leaves can be affected by climatic stresses (e.g. aridity or cold) that may influence the size of the xylem conduits (Gil-Pelegrín et al., 2017). In this sense, many studies showed a higher vulnerability to cavitation in species with wider vessels (Hacke et al., 2000; Tyree, 2003; Hacke et al., 2006; Cai and Tyree, 2010; Jacobsen et al., 2019; Blackman et al., 2023). Embolized xylem conduits leads to hydraulic failure, which is the main cause of plant mortality in response to drought (Tyree and Sperry, 1989). By contrast, many species inhabiting very stressful habitats reduce the diameter of their conduits, achieving a higher resistance to cavitation. This reduction in the conduit diameter results in a lower water transport efficiency (Giordano et al., 1978). However, this decrease may be compensated by increasing the number of conduits (Nardini et al., 2012). When cavitation is caused by freeze-thaw cycles, the same arguments arise. Wider conduits are likewise more vulnerable than small ones because they contain greater dissolved air which can form larger bubbles causing cavitation at lower tensions (Cochard and Tyree, 1990; Sperry and Sullivan, 1992; Lo Gullo and Salleo, 1993; Lemoine et al., 1999; Sevanto, et al., 2012; Zanne et al., 2014; Ni et al., 2022).

Regarding the phloem, other factors rather than climatic stresses might influence the size of its conduits. Sugars, amino acids and other organic metabolites in the sap make sieve tubes a target for some phytophagous insects like aphids (Will et al., 2013). When an aphid stings a sieve tube with its stylet, the plant responds by occluding the sieve plates with callose, turning it into a non-functional conduit (Will and van Bel, 2006). Thus, building more but smaller sieve elements is safer than building a few large conduits (Ewers and Fisher, 1991). Besides, wider sieve elements would need to load more sugars in the source (i.e., the leaf), to generate enough turgor pressure gradient for sap to flow towards the sink organs (Hölttä et al., 2009; Sevanto, 2014).

The scaling relationship between xylem and phloem areas has been explored in several species (Jyske and Hölttä, 2015; Carvalho et al., 2017a; Carvalho et al., 2017b; Kiorapostolou and Petit, 2018; Ray and Jones, 2018; Losada et al., 2022). Most of the studies focus on stems, although Ray and Jones (2018) analyzed petioles in several *Pelargonium* species. Albeit plants tend to present more xylem than phloem, an isometrical scaling has been found in these studies. Nonetheless, most research focuses

129 on a single species. To our knowledge, they do not conduct comparative analyses across
130 different leaf longevities, even in genus level studies. Deciduousness and evergreenness
131 offer different solutions to cope with climatic stresses, which are, in turn, closely related
132 to specific leaf area (SLA) (Sancho-Knapik et al., 2021) (Fig. 1). Similarly, these climatic
133 stresses have been demonstrated to limit the growth of the conduits, even in petioles
134 (Blackman et al., 2023). Since the LSC ultimately depends on the leaf area, the Huber
135 value and the diameter of the conduits, we wonder if the two different leaf habits could
136 develop two different strategies (Fig. 1, models A and B) to reach similar values of LSC.

137 The genus *Quercus* (oaks) offers an excellent system to study the scaling between both
138 conductive tissues, xylem and phloem, besides its ecophysiological implications. Oaks
139 represent a single monophyletic clade with over 400 species occupying very different
140 habitats around the northern hemisphere, from tropical rainforests to cold temperate
141 forests through semideserts and chaparrals. Additionally, oak species exhibit a much
142 broader range not only in terms of leaf area, but also in leaf lifespan compared to other
143 widely studied genera such as *Populus*, *Pelargonium* or *Eucalyptus* (Brocious and Hacke,
144 2016; Ray and Jones, 2018; Blackman et al., 2023). The variability in leaf area can
145 suppose a difference of 70 times between the species with the largest and the smallest
146 leaves (Sancho-Knapik et al., 2021). Finally, phenology ranges from deciduous with a
147 lifespan of just five months up to evergreen species whose leaves can persist over several
148 years (Mediavilla et al., 2008; Harayama et al., 2016).

149 In this study we explore the scaling relationships among the different xylem and phloem
150 traits in petiole cross-sections, leaf area, stomatal conductance, photosynthesis rate and
151 climatic variables in 33 oak species (16 deciduous and 17 evergreen) covering the full
152 range of variation in oaks in terms of leaf area and leaf habit. Based on the information
153 presented thus far (Fig. 1), four aims were addressed: 1) to explore the scaling
154 relationships between conductive tissue structures in petioles —i.e., conductive area and
155 hydraulic diameter of the conduits— and leaf area, for both xylem and phloem; 2) to
156 verify whether deciduous and evergreen species exhibit the same LSC, and to check if
157 deciduous and evergreen oaks follow a different strategy when producing and packaging
158 their conduits to improve their efficiency; 3) in case that two different models are
159 observed, to relate them with climatic variables (mainly aridity and cold) that could be
160 explaining such differences; 4) to assess if the hydraulic architecture resulting from the
161 observed scaling correlates with the physiological demands of the leaf, i.e., stomatal

conductance and photosynthesis rate. We hypothesized that xylem and phloem petiole traits should scale proportionally with leaf area, which ultimately reflects the water demands (stomatal conductance) and export requirements (photosynthesis rate) of the leaf lamina.

MATERIAL AND METHODS

Plant material

In this study, we sampled 22 oak species occurring in the living collection in CITA de Aragón (41°39′N, 0°52′W, 200 m a.s.l., Zaragoza, Spain). In order to get a greater diversity of species and to cover a wider range of leaf areas, we also sampled 11 additional species from Jardín Botánico de Iturrarán 43°15'"N 2°09.3'"W, 200 m a.s.l., Gipuzkoa, Spain).

We sampled five mature leaves of south-exposed branches from 3-5 trees per species. They were sealed in plastic bags and carried to the laboratory. The mid-section of the petiole was cut and stored in 70% ethanol. Then, leaf area (LA) was measured using ImageJ software by scanning the leaf lamina.

Anatomical traits

Petiole sections were dehydrated in a graded ethanol series and subsequently embedded in Paraplast Plus embedding medium (Leica, Richmond, IL, USA). The resulting paraffin blocks were cut in the microtome (HM 350S; MICROM, Walldorf, Germany) to obtain 15-20 µm cross-sections that were stained with saffranine (0.1% w/v), picric acid (0.5% v/v) and AstraBlue (0.1% w/v) after being deparaffinated with Isoparaffin H and rehydrated using a graded ethanol series (100, 95, 90 and 70 %). Then, sections were observed and photographed under a light microscope (OPTIKA B-600TiFL; Optika Microscopes, Ponteranica, Italy) (Fig. 2). We measured the total petiole cross-sectional area (A_{pet}), as well as the conductive area, i.e., the sum of xylem and phloem areas (Fig. 2a). Hereafter, we will use conductive area (A_c) to refer to the sum of the two vascular tissues and we will distinguish between xylem area (A_x) and phloem area (A_p) when treated separately. Besides, we measured the total number of xylem vessel elements and phloem conduits and their mean diameter in three subsamples of the whole conductive

area of each tissue per photograph (Fig. 2b, 2c). In the phloem, our aim was to measure only sieve tubes; however, we cannot claim to have exclusively measured these ones since a visible sieve tube plate (or their identification with callose staining) is necessary to properly identify a sieve tube. We discarded the first brick-shaped cell layers (i.e., the procambium). Medullary rays and cells with a visible nucleus and organelles were also neglected. Finally, very small cells (what we interpret as companion cells or oblique/bevel cuts) as well as the big rounded thick-wall cells in the distal part of the phloem (phloem fibers) were also avoided (Esau, 1939). The rest of the cells were considered as potentially sieve tubes and as such, measured. Afterwards, we calculated the hydraulic diameter for xylem (d_{hx}) and phloem (d_{hp}) using the formula proposed by Sperry et al. (1994):

$$d_h = \frac{\sum_i d_i^5}{\sum_i d_i^4}$$

where d_i is the mean diameter of each conduit measured. Then, we also calculated the ratio of the conductive area normalized by the leaf area following the next formula:

$$\text{For xylem: } XLA = \frac{A_x}{LA \times 10000} \text{ ; For phloem: } PLA = \frac{A_p}{LA \times 10000}$$

Where A_x is the total xylem area, A_p is the total phloem area and LA is leaf area. Leaf area was multiplied by 10 000 to obtain values close to one and to transform units to $cm^2\ m^{-2}$. All measurements were done using ImageJ software. Traits analyzed are compiled in Table 1.

Hydraulic conductivity

We calculated the theoretical hydraulic conductivity of the whole petiole (K_h) as the sum of each conduit conductivity assuming that both types, xylem vessels (K_{hx}) and phloem cells (K_{hp}), follow the Hagen-Poiseuille law (Tyree and Zimmermann, 2002; Hirose et al., 2005; Woodruff, 2014):

$$K_h = \sum_i \frac{d_i^4 \pi \rho}{128 \eta}$$

where ρ is the density of the fluid moving along the conduits at 25 ºC, assuming pure water for xylem (997kg m^{-3}) and a specific sap density dependent of sucrose concentration for phloem (1068 kg m^{-3}) (Jensen et al., 2013), η is the viscosity of the fluid at 25 ºC, pure water for xylem (8.9 x 10^{-10} MPa s) and 1.91 times that value for phloem sap (1.7 x 10^{-9} MPa s) (Thomson, 2006; Jensen et al., 2013); and d_i is the mean lumen diameter of each conduit.

Additionally, we calculated leaf-specific hydraulic conductivity of xylem (LSC):

$$LSC = \frac{K_{hx}}{LA}$$

where LA is leaf area. We also calculated the specific conductivity for xylem (K_{sx}):

$$K_{sx} = \frac{K_{hx}}{A_x}$$

where A_x is the area of xylem in the cross-section of the petiole.

Climatic variables

To get a mean representative value of different climatic variables for each species we followed the same procedure as in Martín-Sánchez et al. (2024). In short, we first downloaded GBIF individual presence points for the 33 species studied, we thinned the data to one presence point per square kilometer using SDMtune R package (Vignali et al., 2020) and we extracted the climatic variables from the WorldClim version 2.1 database (Fick and Hijmans, 2017. WorldClim 2. https://www.worldclim.org). We additionally added an aridity index (AI) calculated as mean annual precipitation (MAP) divided by potential evapotranspiration (PET) (Mencuccini et al., 2019; Peguero-Pina et al., 2020). All individual values were summarized into a mean value for each species. To test our hypothesis whether hydraulic diameters are restrained by climatic factors, we chose variables related to cold and aridity: mean annual temperature (MAT), mean of daily minimum temperatures during the coldest quarter (T_{min}), mean annual precipitation (MAP) and the aridity index (AI).

Leaf gas exchange

We obtained the mean photosynthesis rate (A_N) and stomatal conductance (g_s) of 26 species. For nine species, we measured these parameters using an open gas exchange system (CIRAS-3, PP-Systems, Amesbury, MA, USA) fitted with an automatic universal leaf cuvette (PLC6-U, PP-Systems, Amesbury, MA, USA) in six leaves per species from our living collection. All measurements were conducted under the following standard environmental conditions: CO_2 concentration surrounding the leaf (C_a) of 400 μmol mol^{-1}, leaf temperature of 25 °C, vapor pressure deficit of 1.25 kPa and saturating photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1}. We complemented our own measurements with data for 17 species compiled from other studies (Vaitkus and McLeod, 1995; Nagel et al., 2002; Thadani et al., 2009; Huang et al., 2016; Llusia et al., 2016; Jafarnia et al., 2018; Alonso-Forn et al., 2020; Kar et al., 2021). Assuming a mean value of A_N and g_s for each species and taking into account the mean LA measured for each species, we calculated the theoretical mean photosynthesis rate and stomatal conductance at whole leaf level. ($A_{N,leaf}$ and $g_{s,leaf}$, respectively).

Statistical analyses

First, we tested the potential effect of the garden throughout several analyses of variance (ANOVAs) and linear regressions via mixed models. On the one hand, we performed ANOVAs for each single trait to see how much variance was explained by species and the garden (Table S1). On the other hand, we did linear regressions via mixed models including the garden as a random factor in correlations between pairwise traits, besides a subsequent ANOVA to see how much variance is explained by the random factor. Variance explained by garden in the first ANOVA showed that, for every trait, species accounted for more variance than the garden and in most of the cases species accounted over 60% of variance. Besides, correlations remain significant even when garden is included as a random factor (Table S2). Finally, when we tested differences in the leaf habit, we additionally accounted for garden and the interaction between leaf habit and garden in the ANOVAs. In most of the cases, the interaction resulted to be non-significant, except for three traits (Table S3). Even among those exceptions leaf habit and/or residuals accounted for more variance than the garden. In view of these results, we conclude that correlations are not strongly skewed by an effect of the garden.

Cross-correlations were performed between the different anatomical, hydraulic and physiological traits, assuming a log-log correlation. Alternatively, linear cross-correlations were performed between xylem and phloem hydraulic diameters and climatic variables. Post-hoc analyses of every regression fit were performed using DHARMa R package to test normality, homoscedasticity and outliers (Hartig, 2022). Additionally, we used SMATR R package to check if the scaling relationships were isometric or allometric (Warton et al., 2012). This calculates the slope for the bivariate linear relationship between two variables (after being $\log_{10}$-transformed) following the standardized major axis regression. If the 95% confidence interval of the slope includes the value of 1, isometry cannot be rejected, whereas allometry can be assumed when this confidence interval does not include such value. For anatomical traits and conductivities, we performed slope tests including leaf habit as factor. Finally, for physiological traits, all species were considered together.

RESULTS

The range of variation covered in this study in terms of leaf area goes from 1.9 cm^2 (*Quercus monimotricha*) up to 151 cm^2 (*Quercus macrocarpa*). If species are compared by their leaf habit, significant differences ($P < 0.001$) can be found between deciduous (DEC; 68.7 ± 46 cm^2) and evergreen (EVE; 16.6 ± 15 cm^2) (Fig. 3a). Raw measurements of all the measured diameters are represented as a violin plot to notice the range of variation either between leaf habits or between conductive tissues (Fig. 3b). Hydraulic diameter (d_h) is always significantly wider — either for xylem (d_{hx}) or phloem (d_{hp}) — in deciduous than in evergreen species although the range of variation in xylem vessels diameter (25 ± 7.64 μm in deciduous; 15.8 ± 5.6 μm in evergreen) is higher than in phloem cells diameter (8.19 ± 1.77 μm in deciduous; 6.28 ± 1.42 μm in evergreen). When the conductive area (A_c) is examined (Fig. 3c), deciduous species also display larger xylem and phloem cross-sectional areas (A_x = 297 ± 180 x 10^3 μm^2, A_p = 224 ± 147 x 10^3 μm^2) compared to evergreen species (A_x = 175 ± 121 x 10^3 μm^2, A_p = 128 ± 116 x 10^3 μm^2) (Fig, 2c). Nonetheless, the ratio between cross-sectional areas of the vascular elements in the petiole and LA —i.e., XLA and PLA —, reveals significantly lower values in deciduous species (XLA = 4628 ± 1371, PLA = 3885 ± 2320) compared with evergreen species (XLA = 14207 ± 9038, PLA = 9242 ± 3923) (Fig. 3d), which means that evergreen oaks have a higher conductive area per leaf area. Calculated xylem hydraulic conductivity

(K_{hx}) and xylem specific conductivity (K_{sx}) also present highly significant ($P < 0.001$) differences between deciduous and evergreen species. By contrast, K_{hp} does not show significant differences when leaf habit is considered ($P = 0.902$) (Table 2).

Although the cross-sectional petiole (A_{pet}) area shows a positively significant relationship with LA ($P < 0.001$), the dispersion of the data is quite high, especially in deciduous species (DEC: $R^2 = 0.486$, EVE $R^2 = 0.752$) (Fig. 4a). Conductive area (A_c) presents a strong correlation with LA for both leaf habits ($P < 0.001$, DEC: $R^2 = 0.816$, EVE $R^2 = 0.816$) (Fig. 4b, Table 3).

Xylem and phloem anatomy

We analyze how the conductive area of both tissues, xylem and phloem, as well as the hydraulic diameter of conduits scale with leaf area. In all cases, traits scale positively and significantly ($P < 0.001$) with LA (Fig. 5, Table 3). The larger a leaf is, the larger is the investment in conductive area (Fig. 5a, d) and wider conduits (Fig. 5b, e). However, the relationships are not linear but logarithmic, so that for small leaves, a slight increment in leaf area implies a big increase in both, A_c and d_h, especially in evergreen species. Xylem and phloem also present the same behavior when their ratios are analyzed, i.e., how much A_x and A_p a petiole develops divided by leaf area (Fig. 5c, f). XLA and PLA scale negatively and significantly ($P < 0.001$) with LA either for deciduous or evergreen species (Fig. 5c, f). Evergreen species show a huge heterogeneity in their ratio values for both xylem and phloem for the smallest values of LA. In other words, there is a group of small-leaved evergreen species that invests more in A_x for a specific LA in comparison to large-leaved evergreen leaves. XLA and PLA values above a LA of c.a. 50 cm^2 become asymptotical. The scaling relationships are allometric in all cases (Table 3).

When both conductive areas are correlated, a strong linear relationship ($P < 0.001$) can be appreciated (Fig. S1; $R^2 = 0.716$ for DEC, $R^2 = 0.811$ for EVE). When leaf habit is taken into account, the scaling for deciduous species can be considered isometric (Fig. S1). By contrast, evergreen species present an allometric scaling between A_x and A_p, with more xylem produced than phloem (Fig. S1).

Petiole hydraulic conductivity

342 Calculated hydraulic conductivity of xylem (K_{hx}), i.e., the theoretical capacity of the
343 whole petiole to supply water to the leaf, results to be positively and significantly ($P <$
344 0.001, DEC: $R^2 = 0.724$, EVE $R^2 = 0.560$) correlated with LA (Fig. 6a). Deciduous species
345 with the largest leaves present up to ten-fold higher values of K_{hx} than the evergreen ones
346 with the lowest values (Fig. S2a). For both leaf habits an allometric relationship between
347 K_{hx} and LA is supported (Table 3).

348 The specific conductivity of xylem (K_{sx}) also presents a high significance ($P < 0.001$) in
349 both groups in relation with LA, although correlations are much weaker in comparison to
350 K_{hx}, especially for evergreen species (DEC: $R^2 = 0.415$, EVE: $R^2 = 0.281$) (Fig. 6b, Table
351 3). Deciduous species present significant higher values of K_{sx} than evergreen ones (Fig.
352 S2b). In this case, isometry cannot be rejected for either deciduous or evergreen species
353 (Table 3). Leaf-specific conductivity (LSC) is significantly higher in deciduous species
354 than evergreen ones ($P < 0.001$) (Fig. S2c).

355 Calculated phloem hydraulic conductivity (K_{hp}) is in all cases much lower than for xylem
356 with weaker or non-significant correlations with LA (DEC: $R^2 = 0.276$, $P = 0.06$; EVE:
357 $R^2 = 0.036$, $P = 0.55$) (Table 3, plot not shown). It is over 100 times lower than the K_{hx} on
358 average for deciduous species (data not shown). Differences among evergreen species are
359 less remarkable, with a K_{hx} c.a. 20 times higher than K_{hp} on average and some specific
360 individuals with a similar conductivity for both conductive tissues (data not shown).

361 When XLA is compared with the K_{sx} (Fig. 7a), it can be noticed how deciduous species,
362 whose leaves are larger, hardly present variation in their XLA values. By contrast they
363 display a wide range of values in their K_s. Conversely, evergreen species show a wide
364 range of variation in their XLA values without an apparent increase in their K_{sx}, with the
365 exception of *Q. costaricensis*, which is, in turn, among the species with the largest leaves
366 within evergreen oaks.

367 Similarly, in the comparison between XLA with the respective d_{hx} (Fig. 7b), it can be seen
368 how individuals tend to contribute mainly to one axis depending on their leaf habit. This
369 is, deciduous species basically present much higher range of variation in d_{hx} than in XLA.
370 By contrast, evergreen oaks present a larger variation in XLA than in d_{hx}. Both increments,
371 either in d_{hx} or in XLA leads to an improvement in the LSC, although species that increase
372 their d_{hx}, represented by deciduous oaks, improve their LSC more than evergreen oaks
373 that increase their xylem area.

374

375 *Climatic correlations*

376 Mean annual precipitation reveals a significant relationship with d_{hx} for evergreen species
377 (P = 0.02) but no significance is found in deciduous (P = 0.07). Taking into account the
378 potential evapotranspiration, i.e., comparing the aridity index (AI) with d_{hx} improves the
379 relationships. Aridity index shows positive correlation with d_{hx} for both deciduous (P =
380 0.03; R^2 = 0.249) and evergreen species (P = 0.02; R^2 = 0.274) (Fig. S3). The smallest
381 hydraulic diameters are displayed in the most xeric species. Regarding temperature, MAT
382 does not seem to be significantly related to d_{hx} either for deciduous (P = 0.24) or evergreen
383 species (P = 0.91). Conversely, T_{min} was only compared for evergreen species since
384 deciduous oaks lack leaves during winter. The correlation did not present significance (P
385 = 0.37; Fig. S4).

386

387 *Relationships between vascular traits, stomatal conductance and photosynthesis net rate*

388 When the main attributes of the xylem in the petiole are correlated with the stomatal
389 conductance at leaf level ($g_{s,leaf}$), significant relationships can be appreciated in all cases
390 (P < 0.001) (Fig. 8). There is a significant increase in $g_{s,leaf}$ as A_x becomes larger with an
391 allometric relationship (R^2 = 0.512) (Fig. 8a, Table 4). For a given value of A_x, deciduous
392 species tend to present higher values of $g_{s,leaf}$ than evergreen ones. Stomatal conductance
393 also increases allometrically as d_{hx} becomes wider, but with a steeper slope (R^2 = 0.586)
394 (Fig. 8b, Table 4). Likewise, deciduous species usually present higher values of $g_{s,leaf}$ for
395 the same d_{hx} than evergreen ones. Once again, K_{hx} also scaled allometrically (Fig. 8c,
396 Table 4) and deciduous species have higher values than evergreen species on average.

397 When the same xylem traits are correlated with the photosynthesis net rate, exactly the
398 same trends arise (Table 4). The $A_{N,leaf}$ appears to be related to A_x (Fig. 8d, P = 0.001, R^2
399 = 0.591), d_{hx} (Fig. 8e, P < 0.001, R^2 = 0.671) and K_{hx} (Fig. 8f, P < 0.001, R^2 = 0.786).

400 Photosynthesis net rate at leaf level ($A_{N,leaf}$) is significantly correlated with phloem
401 anatomical traits (P < 0.001) (Fig. 9, Table 4). $A_{N,leaf}$ increases with larger A_p (R^2 = 0.561)
402 (Fig. 9a) and wider d_{hp} (R^2 = 0.509) (Fig. 9b) being on both cases an allometric
403 relationship. Deciduous species tend to present higher photosynthesis net rates at leaf

404 level than evergreen ones. Finally, the relationship between $A_{N,leaf}$ and K_{hp} is barely
405 significant (P = 0.043) with a very week correlation (R^2 = 0.130) (plot not shown).

406

407 DISCUSSION

408 *Anatomical traits scale with leaf area*

409 In the *Quercus* species studied, we found associations between the anatomical traits of
410 the petioles and leaf area. First, the cross-sectional area of the petiole displays a rather
411 scattered association with LA (yet significant). In general, there is an allometric
412 relationship for deciduous species, whereas evergreen oaks are better adjusted to an
413 isometric scaling. As we first hypothesized, both hydraulic diameter and conductive area
414 scale with LA, either as a whole (A_c) or separating between xylem (A_x) and phloem (A_p).
415 This means that the larger the leaf, the greater the ability for bulk transport of water and
416 carbohydrates. Increasing the conductivity can be achieved either by increasing the
417 number of conduits, by producing wider conduits or by a combination of both strategies.
418 Nonetheless, the scaling becomes weaker in larger leaves.

419 The observed asymptotic response may reflect the different beforementioned constraints
420 that can compromise the functionality of the conducting tissues. The trade-off between
421 support and conduction functions of petioles could be explaining the constraint to produce
422 linearly larger conductive areas in larger leaves. The hydraulic diameter cannot scale
423 infinitely either. In the xylem, wider conduits are more susceptible to cavitation by both
424 drought and freezing. For phloem, leaves with wider sieve tube elements would require a
425 sugar production commensurate with the size of such conduits to generate an adequate
426 turgor pressure gradient to transport the phloem sap. Otherwise, allocation of sugars
427 would be hindered. Sieve elements differences can be found depending on the organ, age
428 and life-form. Thus, stems usually present the widest ones because of the presence of
429 secondary phloem, in contrast with organs with primary phloem such as leaves or petioles
430 (Woodruff, 2014; Prislan et al., 2019). Mature trees also present wider sieve elements
431 than seedlings or saplings (Kopanina et al., 2022). Finally, vines usually develop wider
432 sieve elements than free-standing plants, since they do not need as much support tissue
433 as a tree (Ewers and Fisher, 1991; Losada et al., 2022). Despite these differences,
434 interspecific variation of sieve element diameter is lower than for xylem vessel diameter,
435 which agrees with our results. Thus, the limitations imposed to phloem seem to be more

restrictive than those imposed to xylem. This makes sense if we consider that maintaining the proper function of phloem is more critical than xylem for several reasons. First, phloem sap flows during the whole day, night included, in contrast to xylem flow, which reaches the highest values when stomata are opened during the day. Second, phloem must maintain a constant turgor to achieve a steady flow since either an excessive viscosity or a loss of turgor level will hinder the sap flow (Lang, 1978).

The scaling relationship between xylem and phloem areas has been also explored in several studies (Table 5), which find an isometric scaling between A_x and A_p. Nonetheless, most of these studies only focus on single species. Our work clearly improves this by exploring the scaling relationship in a great number of species, closely related but different enough in leaf habit and climatic ranges. Our data support an isometric scaling in the case of deciduous species, but an allometric scaling between xylem and phloem in evergreen species, favoring more production of xylem than phloem area (Fig. S1), which reflects the higher values of XLA in evergreen species (see next section for further details). A scaling relationship between the conductive areas in any case should be expected, since both tissues are originated from the same meristematic tissue, i.e., the procambium. In addition, despite having very different function, xylem and phloem are interconnected. The main hydric relationship relies on xylem supplying water to load phloem companion cells and sieve tubes according to a lateral water potential gradient between both tissues. The flux of sugars depends on the product of water flux and sugar concentration. Since the sugar concentration declines with distance from the leaf, water flux must increase to keep the sap flux steady. In other words, there is an influx of water from the xylem throughout the transport phloem to compensate for the lower sugar concentrations. The balanced interaction between xylem and phloem is an essential requirement for long-distance transport (Dinant and Lemoine, 2010; Sevanto, 2014).

Concerning the hydraulic conductivity after applying Hagen-Poiseuille law, we reported an improvement in xylem K_h with LA, due to the combination of both a larger A_x and wider d_h. This increment in xylem efficiency is still reflected even after removing the effect of developing more A_x due to larger leaf areas, i.e., the K_{sx}. However, this is not the case for phloem, where the dispersion of the data is much higher (Table 3). Here, solely diameter of the phloem cells does not seem to predict the actual hydraulic conductivity of phloem by itself, probably due to a mix of cellular types in the measurements. In addition, some other factors related to the nature of sieve plates, such as the number of

pores, diameter of such pores and even number of plates per sieve element, are likely to modulate the hydraulic conductivity of phloem.

Deciduous and evergreen oaks follow different strategies producing and packaging their conduits

The main differences between deciduous and evergreen oak species arise when we compare the conductive area standardized by LA (i.e., the XLA) with the K_{sx} and d_{hx} (Fig. 7). In this scenario, the range of variation in both K_{sx} and d_{hx} mainly corresponds with deciduous species, whereas the range of variation in XLA mostly corresponds with evergreen species. The larger LA of deciduous oaks requires a higher water supply which is, in turn, reflected by a higher photosynthetic rate and stomatal conductance compared to evergreen species. Thus, deciduous species display up to an order of magnitude higher K_{sx} values than evergreen oaks. An increase in K_{sx} can be achieved either by reducing the xylem area or by widening the xylem vessels for the same size and number of vessels, which ultimately increases K_{hx}. Since the xylem area increases with LA, this increase in K_{sx} in deciduous species can only be modulated by an increase in the diameter of the xylem vessels. By contrast, evergreen oaks hardly present range of variation in their K_{sx} values, with the exception of *Q. costaricensis*, the evergreen oak with the widest vessels in this study.

Subsequently, we compared d_{hx} with XLA, but this time transforming K_{sx} into a more physiologically meaningful variable, that is LSC, which links the capacity of xylem to transport water with the leaf water demands (Mencuccini et al., 2019). In this correlation (Fig. 7b), deciduous oaks always present low XLA values, close to or lower than one, but they display a wide range of variation in their d_{hx}. Conversely, evergreen oaks exhibit a wider variation in their XLA values but narrower d_{hx} values. In other words, deciduous species tend to produce wider conduits to improve their xylem hydraulic conductivity for a given leaf area, whereas evergreen species choose to increase their A_x for the same leaf area over the d_h.

This dichotomous strategy between deciduous (Fig. 1, Model A) and evergreen (Fig. 1, Model B) oaks could be directly related with both, their leaf life spans and the climatic niches they occupy. First, deciduous leaves only have to keep functional for a few months (typically 6-9 months). Thus, they can take a riskier but, simultaneously, a more effective

—showed by high LSC values— and a cheaper strategy (Ni et al., 2022). On the other hand, evergreen species, whose leaves must remain productive for longer periods, tend to follow a safer strategy at the expense of a more costly investment (Hacke et al., 2000). Nonetheless, this investment in larger A_x also increases the LSC in those species with high values of XLA, partly counterbalancing their lower K_{hx} values and reaching efficiencies close to deciduous species. Besides, this safer strategy could be the main contributor to the allometry found in xylem for evergreen species in comparison to the isometry that most studies find and is also present in our deciduous species.

Second, deciduous oaks considered in this study are mainly represented by species occupying temperate forests. These habitats rarely present stressful conditions (drought and/or cold) during the lifespan of the leaves (Peguero-Pina et al., 2016). Hence, it is reasonable to think that deciduous oaks could afford more efficient vessels at the expense of more vulnerability. Accordingly, most of the evergreen oak species (with the exception of some tropical ones; e.g., *Q. costaricensis*) must cope with at least one stressful period during the year (typically a drought period), and even two in the case of Mediterranean species (summer drought and winter cold) (Martín-Sánchez et al., 2022). Therefore, it is justifiable to consider that these species choose a conservative strategy for building their conductive tissues. Furthermore, deciduous oaks occupying extra-temperate habitats with stressful periods such as the Mediterranean Basin (e.g., *Q. faginea* and *Q. ithaburensis*) or winter-dry temperate climates in Mexico (e.g., *Q. crassipes*) present the smallest values of both leaf area and hydraulic diameter among deciduous oaks, suggesting the reduction of xylem vessels in environmental restrictive habitats. Indeed, aridity index shows correlation with d_{hx} for both deciduous and evergreen species. The more xeric the climate is, the narrower the xylem vessels are. This relationship between drought and vessel size has been widely reported by numerous authors in stems, branches and leaves, resulting in a trade-off between efficiency and safety (Hajek et al., 2014; Pivovaroff et al., 2016; Schreibet et al., 2016; Barotto et al., 2017). It has also been recently found in petioles by comparing XLA and resistance to cavitation in several *Eucalyptus* species (Blackman et al., 2023). Likewise, this compensation of improving the hydraulic conductivity by increasing the conductive area over the diameters of the conduits has been also reported in stems of several species (Nardini et al., 2012) but, to our knowledge, it has never been attributed to leaf habit in any case.

We demonstrate the presence of two models for producing and packaging the conduits, and we also prove the relationship between aridity and hydraulic diameter. However, we did not find significant correlation between cold, here represented by the WorldClim2 variable "mean of daily minimum temperatures during the coldest quarter", and d_{hx} in evergreen oaks. Nonetheless, cavitation induced by winter cold is caused by freeze-thaw cycles, a climatic variable for which global-scale data are not available. The lack of significance is mainly due to two species: *Q. semecarpifolia* and *Q. engleriana*. These evergreen species present a wide range of distribution in Asia, in habitats that present a complex orography, resulting in very different climatic conditions. A detailed study in their natural habitats along altitudinal and climatic gradients, measuring the daily temperatures, might reveal a reduction in d_{hx} in those sites where trees have to withstand more frequent freeze-thaw cycles. Other species, such as *Q. chrysolepis* and *Q. monimotricha*, for instance, the two species with the narrowest vessels, can be found in very high-altitude habitats, where they are exposed to recurrent frosts during the coldest months. Thus, according to the leaf economic spectrum, these species would not recover the investment in case such expensive leaves died earlier due to a hydraulic failure. By contrast, evergreen species with the widest vessels (e.g. *Q. costaricensis*, *Q. virginiana*) occupy tropical or subtropical habitats with the absence of strong and frequent frosts.

Anatomy of petioles accommodates physiological demands

Our data supports a strong correlation between the petiole anatomical traits of both, xylem and phloem, and the estimated g_s and A_N at leaf-level. The strongest relationships are found between xylem traits and $A_{N,leaf}$, albeit xylem-$g_{s,leaf}$ correlations shows similar statistical power. Even though Figures 8 and 9 represent deciduous and evergreen species in different colours, the aim is not to see differences in leaf habit but explore the anatomical architecture in response to the physiological demands of the leaf lamina. This link function-structure has been proposed to be mediated throughout several physiological processes, such as water potential, hydraulic conductance, turgor pressure or sugar concentration (Hölttä et al., 2010). These factors would have an effect on the ontogeny and development of the cells in a tissue (Cosgrove, 1993).

Relationships between xylem area in the petiole and leaf transpiration were proposed more than one hundred years ago by Salisbury (1913). However, this author suggested

565 that the nature of the conduits —i.e., number and size— should receive more attention.
566 Here, we explore not only such relationship between A_x and $g_{s,leaf}$, but also the size of the
567 conduits and the calculated K_{hx}, which all resulted to be highly correlated with $g_{s,leaf}$.
568 Brocious and Hacke (2016), presented a study among different *Populus* hybrids where no
569 differences among several clones were found, however, when all leaves were analyzed
570 together, they found similar trends to our findings for A_x and K_{hx} in relation to g_s,
571 suggesting that 'lamina size is constrained by the transport capacity of the vascular tissue
572 in the petiole'. Concerning the scaling relationship, our results show an allometry in all
573 cases. In this regard, Zhong et al. (2020) also found allometric scaling in 53 woody
574 species between xylem area in the midrib and the number of stomata in the leaf lamina,
575 but they reported an isometric scaling of leaf area and total stomatal area. Nonetheless, it
576 must not be disregarded that stomatal conductance is only showing the capacity of
577 stomata to release water to atmosphere, but the transpiration rate is the variable actually
578 measuring water losses in leaves by taking into account the vapor pressure deficit (VPD).
579 In this context, part of the scatter observed in the association between petiole xylem traits
580 and $g_{s,leaf}$ might be attributed to adaptations to different VPD levels during the growing
581 season.

582 A higher photosynthetic rate is related to a larger xylem hydraulic conductance because
583 of a greater water usage (Brodribb and Field, 2000; Hölttä et al., 2010). The largest leaves
584 among our species correspond with deciduous species, and they present a higher $A_{N,leaf}$
585 compared to evergreen species. However, when A_N —expressed in m^2— is compared, no
586 significant differences linked to leaf habit are found (Peguero-Pina et al., 2017). In this
587 case, taking into account the total photosynthesis rate at leaf level is more logical than
588 standardizing it for a given area because a petiole must have an anatomical structure able
589 to export the sucrose produced by the leaf. Sucrose is the most abundant photosynthate
590 transported by sieve elements, but viscosity of a sucrose solution increases exponentially
591 with increasing concentration (Morison, 2002). Furthermore, the viscosity of the sap is
592 one of the main factors that limit phloem transport, since the more viscous the solution,
593 the lower the flow rate (Lang, 1978; Sevanto, 2014). To deal with this disadvantage, plants
594 can choose between two strategies. On the one hand, sink organs could lower their sugar
595 concentration, increasing the source-sink concentration gradient. On the other hand, they
596 could develop wider sieve tubes, since an increment in the radius of a sieve tube should
597 improve the hydraulic conductivity to the fourth power. This latter strategy seems to be

598 more feasible for the plant (Hölttä et al., 2009; Sevanto, 2014). Thus, the largest leaves in
599 oak species, which are in turn the ones which produce more sucrose, would need wider
600 sieve tubes to avoid a depleted flow rate caused by an excessive viscosity.

601

602 CONCLUSION

603 The conductive tissues in the petiole scale allometrically with leaf area, which ultimately
604 reflects the demands on the leaf. Xylem and phloem present a very similar pattern in their
605 scaling both for conductive areas and for the diameters of their cells. Although increasing
606 the diameter of the conduits would imply a greater improvement in the hydraulic
607 conductivity than increasing the conductive area, it also results in a riskier strategy. For
608 this reason, a coordinated scaling between both alternatives is required depending on the
609 habitat occupied by the species. For example, species inhabiting arid habitats tend to have
610 narrower conduits than those species occupying cool nemoral habitats.

611 We find that oaks with different leaf habits tend to improve their hydraulic conductivities
612 with two contrasting approaches. Deciduous species opt to produce wider vessels for the
613 same conductive and leaf areas compared to evergreen oaks. Conversely, evergreen
614 species choose to increase their conductive area over the diameter of the conduits.

615 Most studies inquiring into the scaling between xylem and phloem find isometric
616 relationships. Deciduous oaks exhibit the same isometric pattern. However, evergreen
617 species present an allometric scaling, producing more xylem than phloem, which agrees
618 with their safer strategy.

619 Phloem is more constrained than xylem in increasing the diameter of its main conduits.
620 This is probably related to the very different functionality of both tissues. While xylem
621 mainly responds to water demands during the photosynthesis, phloem is responsible for
622 maintaining a proper balance of sugars, hormones and other metabolites throughout the
623 plant and throughout the day.

624 The structure of the conductive tissues straightly corresponds with leaf demands. Xylem
625 area, vessel size and hydraulic conductivity in the petiole are correlated with both
626 photosynthesis net rate and stomatal conductance at leaf level. Phloem anatomy also
627 relates to photosynthesis rate.

628

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in https://doi.org/https://doi.org/10.5281/zenodo.12730852.

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TABLES

Table 1. Appendix of traits measured in this study; their abbreviations and units.

Parameter	Abbreviation	Unit
Leaf Area	**LA**	cm^2
Petiole area	**A_{pet}**	μm^2
Conductive area	**A_c**	μm^2
Xylem area	**A_x**	μm^2
Hydraulic diameter of xylem	**d_{hx}**	µm
Ratio xylem area/leaf area	**XLA**	$cm^2\ m^{-2}$
Hydraulic conductivity of xylem	**K_{hx}**	Kg m $Mpa^{-1}\ s^{-1}$
Specific conductivity of xylem	**K_{sx}**	Kg m^{-1} $Mpa^{-1}\ s^{-1}$
Phloem area	**A_p**	μm^2
Hydraulic diameter of phloem	**d_{hp}**	µm
Ratio phloem area/leaf area	**PLA**	$cm^2\ m^{-2}$
Hydraulic conductivity of phloem	**K_{hp}**	Kg m $Mpa^{-1}\ s^{-1}$
Photosynthesis net assimilation per leaf	**$A_{N,leaf}$**	µmol $CO_2\ s^{-1}$
Stomatal conductance per leaf	**$g_{s,leaf}$**	mmol $H_2O\ s^{-1}$
Mean annual temperature	**MAT**	ºC
Mean annual precipitation	**MAP**	mm
Mean of daily minimum temperatures during the coldest quarter	**T_{min}**	ºC
Aridity Index	**AI**	Dimensionless

Table 2. Percentage of variance explained by leaf habit (deciduous and evergreen) according to the ANOVA performed for each trait measured individually. Leaf traits notation as in Table 1. Significance level is showed with asterisks (*** < 0.001, ** = 0.001-0.01, * = 0.01-0.05, n.s. > 0.05).

Trait	Leaf Habit		Residuals
LA	37.43	***	62.57
A_{pet}	5.4	n.s.	94.6
A_c	14.12	*	85.88
A_x	13.97	***	86.03
d_{hx}	32.51	***	67.49
XLA	32.95	***	67.05
K_{hx}	18.22	***	81.78
K_{sx}	28.61	***	71.39
A_p	11.72	***	88.28

d_{hp}	25.17	***	74.83
PLA	29.23	***	70.77
K_{hp}	0.88	n.s.	99.12

Table 3. Scaling exponents of each leaf habit (deciduous and evergreen) separately for standardized major axis (SMA) regressions. All variables were $\log_{10}$ transformed. The scaling relationship (isometry or allometry) was selected taking into account if the 95% confident interval of the slope includes the value 1 (isometry) or not (allometry). All correlations are significant ($P < 0.001$) but K_{hp} with LA. Leaf traits notation as in Table 1. Differences in the slope and elevation between deciduous (DEC) and evergreen (EVE) species are shown with asterisks ($P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *, NS = No significant).

			Deciduous			Evergreen			DEC vs EVE	
y	x	Figure	Slope	Scaling relationship	R^2	Slope	Scaling relationship	R^2	Slope	Elevation
A_{pet}	LA	Fig. 4a	0.73	Allometry	0.486	0.9	Isometry	0.752	*	***
A_c	LA	Fig. 4b	0.71	Allometry	0.816	0.79	Allometry	0.816	NS	***
A_x	LA	Fig. 5a	0.74	Allometry	0.874	0.76	Allometry	0.651	NS	***
d_{hx}	LA	Fig. 5b	0.33	Allometry	0.507	0.37	Allometry	0.563	NS	NS
XLA	LA	Fig. 5c	-0.4	Allometry	0.579	-0.59	Allometry	0.429	*	*
A_p	LA	Fig. 5d	0.75	Allometry	0.599	0.87	Allometry	0.69	NS	***
d_{hp}	LA	Fig. 5e	0.24	Allometry	0.2	0.24	Allometry	0.443	NS	*
PLA	LA	Fig. 5f	-0.63	Allometry	0.439	-0.56	Allometry	0.24	NS	NS
A_p	A_x	Fig. S1	1.01	Isometry	0.716	1.15	Allometry	0.811	NS	NS
K_{hx}	LA	Fig. 6a	1.57	Allometry	0.724	1.66	Allometry	0.56	NS	NS
K_{sx}	LA	Fig. 6b	1	Isometry	0.415	1.18	Isometry	0.281	NS	NS
K_{hp}	LA	Plot not shown	1.13	Isometry	0.276	1.61	Isometry	0.036	NS	NS

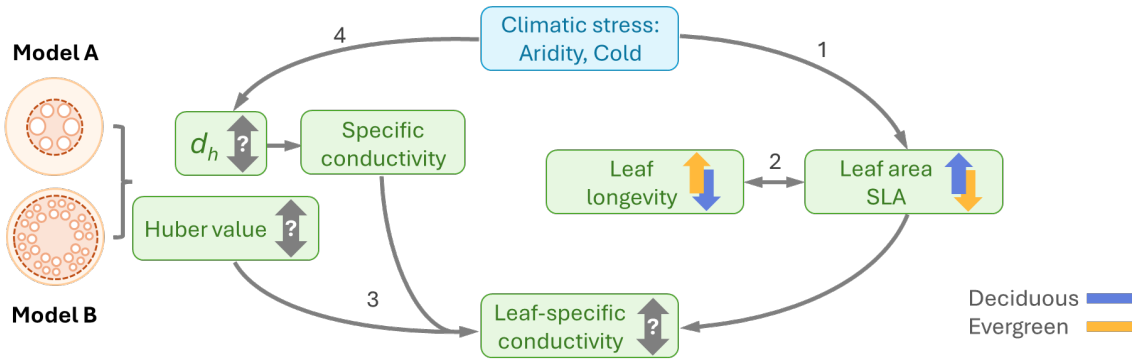
Table 4. Scaling exponents of physiological traits for standardized major axis (SMA) regressions. All variables were $\log_{10}$ transformed. Leaf traits notation as in Table 1. Every correlation is significant ($P < 0.05$) and scale allometrically (slope significantly different of 1).

y	x	Figure	Slope	Scaling relationship	R^2
A_x	$g_{s,leaf}$	Fig. 8a	0.61	Allometry	0.512
d_{hx}	$g_{s,leaf}$	Fig. 8b	0.38	Allometry	0.586
K_{hx}	$g_{s,leaf}$	Fig. 8c	1.86	Allometry	0.684
A_x	$A_{N,leaf}$	Fig. 8d	0.63	Allometry	0.591
d_{hx}	$A_{N,leaf}$	Fig. 8e	0.39	Allometry	0.671
K_{hx}	$A_{N,leaf}$	Fig. 8f	1.91	Allometry	0.786
A_p	$A_{N,leaf}$	Fig. 9a	0.73	Allometry	0.561
d_{hp}	$A_{N,leaf}$	Fig. 9b	0.23	Allometry	0.509

Table 5. References of studies exploring the scaling relationship between xylem and phloem conductive areas, specifying the species and organs studied as well as the slope and the nature of the scaling found, either isometry or allometry.

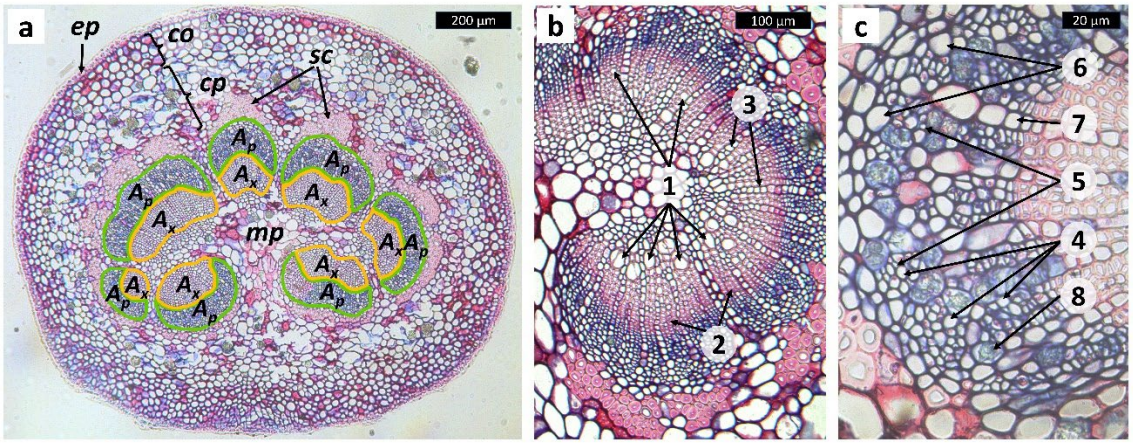
Reference	Species	Organ	Slope	Scaling relationship
Jyske and Hölttä 2015	*Picea abies*	Stem	0.93	Isometry
Carvalho et al. 2017a	*Populus* × *canescens*	Leaf, petiole	0.96	Isometry
Carvalho et al. 2017b	*Ginkgo biloba*	Leaf	0.91	Isometry
Kiorapostolou and Petit 2019	*Fraxinus ornus*	Stem	0.96	Isometry
Ray and Jones 2018	*Pelargonium* (11 spp.)	Petiole	0.87	Isometry

964 **FIGURES**



965

966 **Fig. 1.** Scheme of the traits that ultimately modulate the leaf-specific conductivity (LSC)
967 of the petiole. Thick colored arrows show the tendency of each leaf habit (deciduous in
968 blue and evergreen in orange) to have larger (upward arrow) or smaller (downward arrow)
969 values for leaf longevity, leaf area and specific leaf area (SLA). Thick grey arrows
970 represent the unknown relationships we aim to explore in this study. Two anatomical
971 models are proposed: model A assumes that LSC can be improved increasing the
972 hydraulic diameter (d_h), whereas model B assumes that for the same cross-section of
973 petiole, a similar LSC could be reached by increasing the conductive area with a smaller
974 d_h. References that support the proposed relationships are: 1) Sancho-Knapik et al.
975 (2021); 2) Mediavilla et al. (2008) and Kikuzawa et al. (2013); 3) Mencuccini et al.
976 (2019). 4) Blackman et al. (2023).

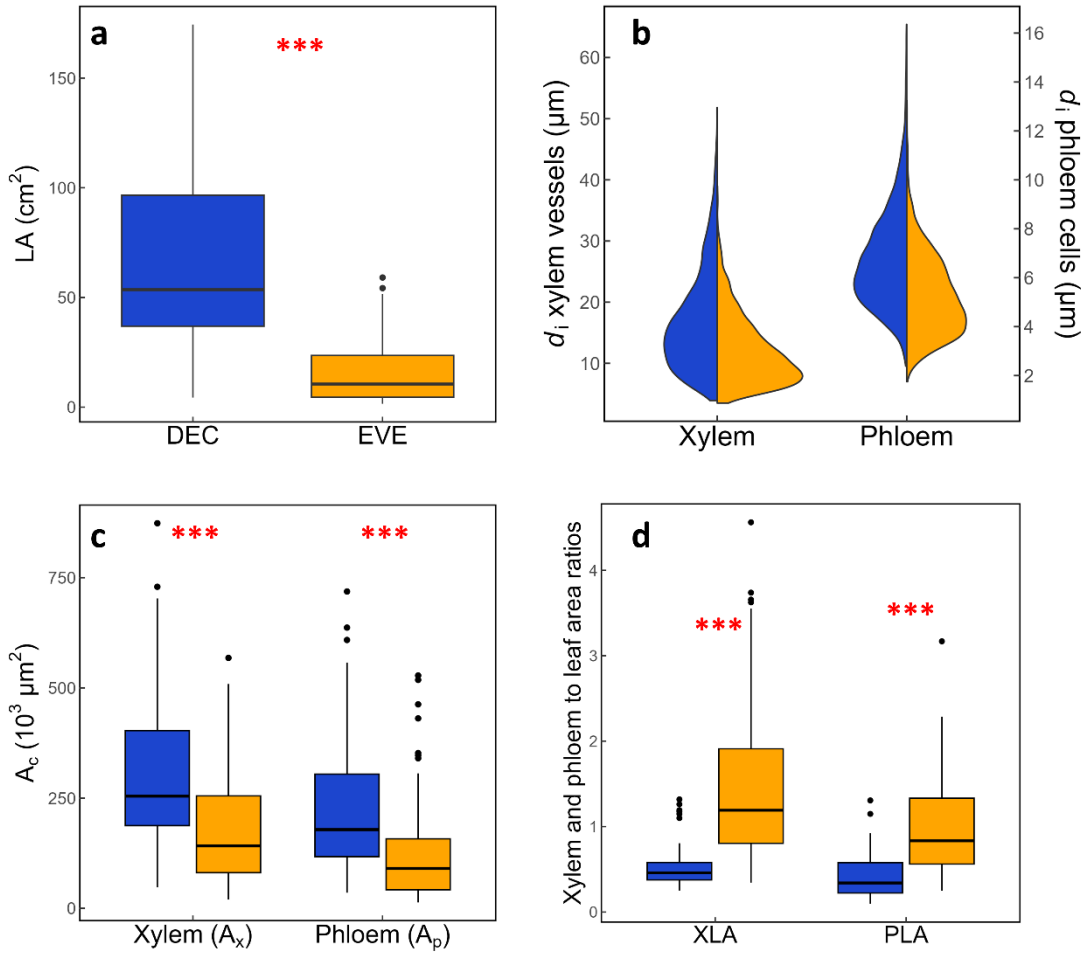


977

978 **Fig. 2.** Histological cross-section of *Quercus agrifolia* petiole. **(a) General scheme** of the
979 whole petiole with the main tissues: epidermis (ep), collenchyma (co), cortical
980 parenchyma (cp), sclerenchyma (sc), medullary parenchyma (mp) and the conductive
981 tissues, measured in this study: xylem (A_x, highlighted in yellow) and phloem (A_p,
982 highlighted in green). **(b) Magnified view of xylem** with its main cellular types: xylem

983 vessels (1; measured in this study), tracheids (2) and parenchymatic medullary rays (3).
984 **(c) Detailed view of phloem** with its main cellular types: potential sieve tubes (4;
985 measured in this study), potential companion cells (5), phloem fibers (6), medullary rays
986 (7) and parenchyma (8).

987



988

989 **Fig. 3.** Distribution of the main traits measured: a) Boxplot of the leaf area (LA), b) Violin
990 plot of the diameters (d_i) of the conduits (vessels for xylem and sieve tubes for phloem),
991 c) Boxplot total conductive area (A_c) in the petiole, and d) Xylem and phloem to leaf area
992 ratios, i.e., conductive area divided by LA. Red asterisks show significant ($P < 0.001$)
993 differences between leaf habits (blue, deciduous; orange, evergreen). Note the double Y
994 scale in panel b).

995

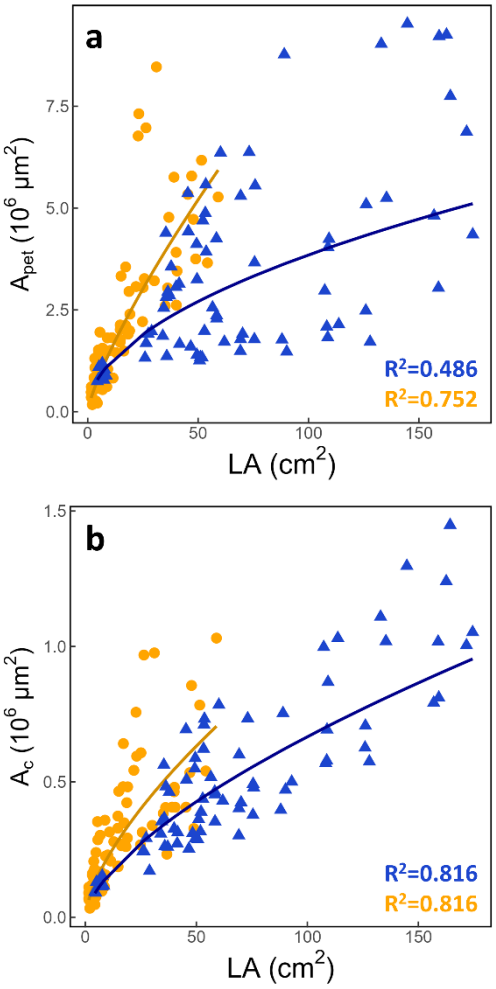
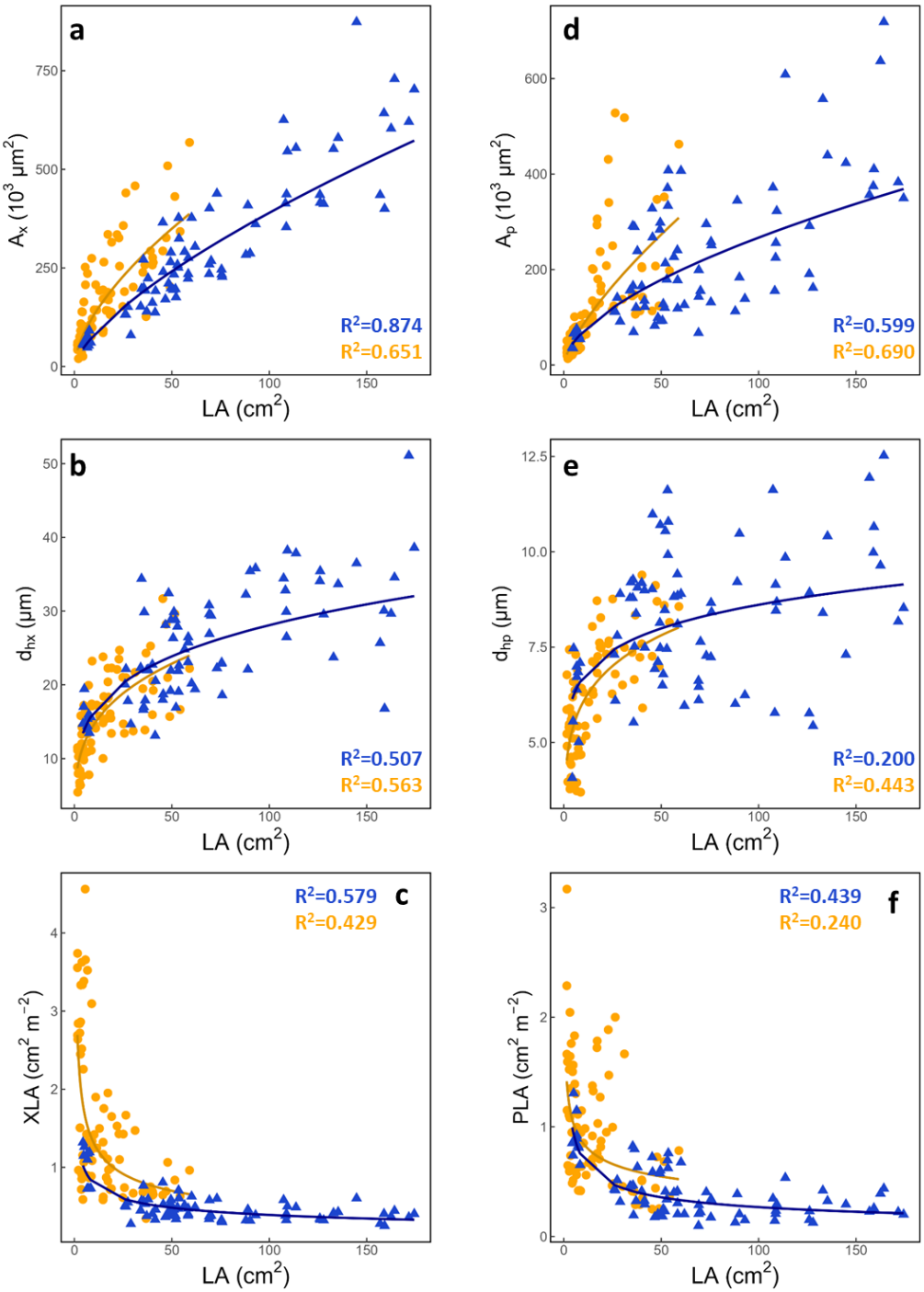
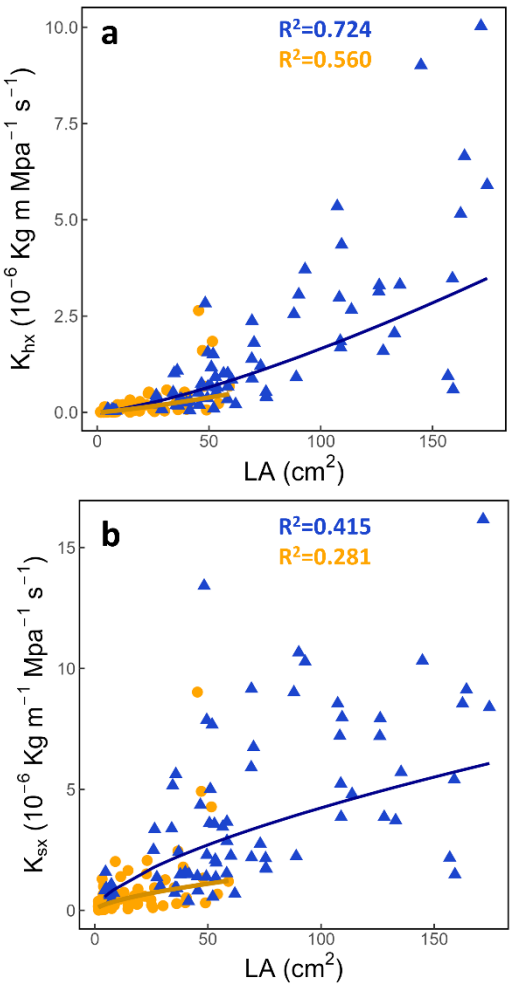


Fig. 4. Scaling relationships of a) leaf area (LA) with cross-sectional petiole area (A_{pet}) and b) LA with conductive area (Ac, the sum of xylem and phloem areas) for deciduous (blue triangles) and evergreen (orange dots) species. Each point represents one individual measure. Colored continuous lines represent the best fit for each leaf habit separately. All regressions are highly significant ($P < 0.001$).



1002

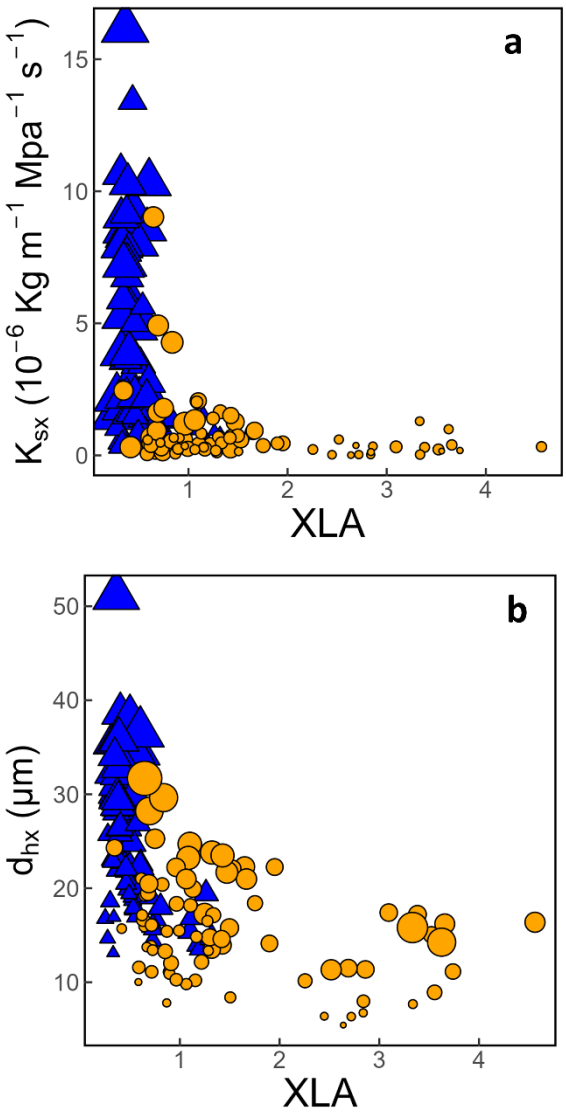
1003 **Fig. 5.** Correlations related to xylem (panels a-c) and phloem (panels d-f) anatomy for
1004 deciduous (blue triangles) and evergreen (orange dots) species between leaf area (LA)
1005 and: a) xylem cross-sectional area (A_x), b) xylem hydraulic diameter (d_{hx}), c) xylem cross-
1006 sectional area divided by LA (XLA), d) phloem cross-sectional area (A_p), e) phloem
1007 hydraulic diameter (d_{hp}) and f) phloem cross-sectional area divided by LA (PLA). Each
1008 point represents one individual measure. Colored continuous lines represent the best fit
1009 for each leaf habit separately. All regressions are highly significant ($P < 0.001$).



1010

1011 **Fig. 6.** Correlations related to a) xylem hydraulic conductivity (K_{hx}) and b) specific
1012 hydraulic conductivity (K_{sx}) for deciduous (blue triangles) and evergreen (orange dots)
1013 species. Each point represents one individual measure. Colored continuous lines represent
1014 the best fit for each leaf habit separately. All regressions are highly significant ($P < 0.001$).

1015



1016

1017 **Fig. 7.** Correlations between a) XLA and the xylem specific conductivity (K_{sx}) showing
1018 the leaf area as the relative size of the symbols (larger symbols represent larger leaf areas);
1019 and b) between the XLA and the xylem hydraulic diameter (d_{hx}) showing the leaf specific
1020 conductivity (LSC) as the relative size of the symbols (larger symbols show higher LSC
1021 values). Leaf habit is represented by deciduous in blue triangles and evergreen species in
1022 orange dots. Each point represents one individual measure.

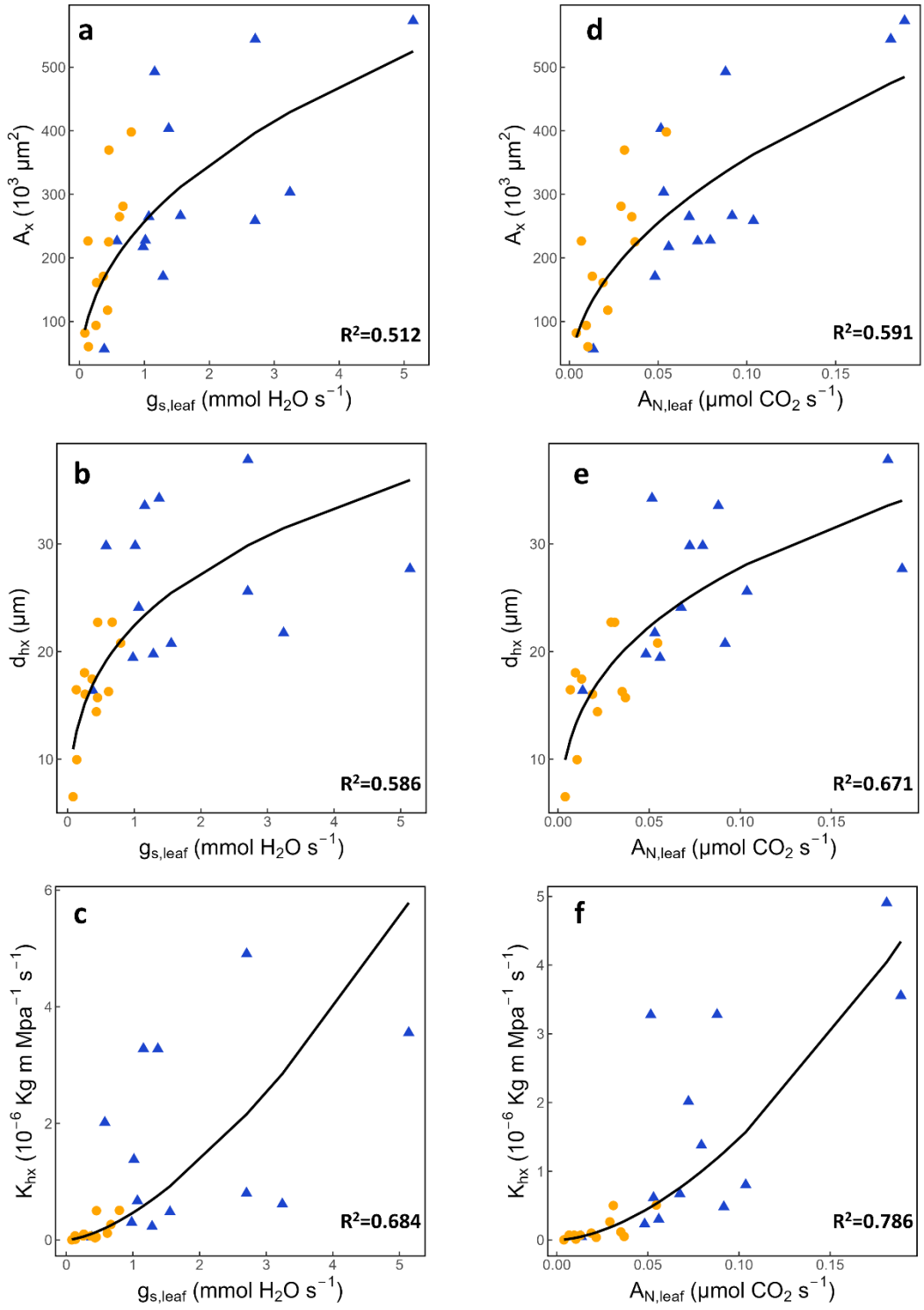
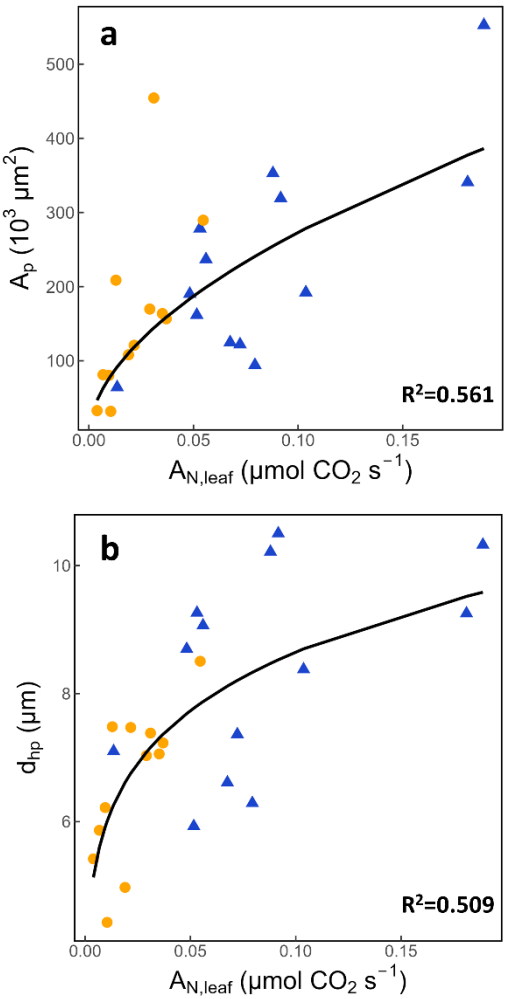


Fig. 8. Relationships between xylem traits (cross-sectional area (A_x), hydraulic diameter (d_{hx}) and calculated hydraulic conductivity (K_{hx})), stomatal conductance ($g_{s,leaf}$) and photosynthesis net rate ($A_{N,leaf}$). Blue triangles are deciduous and orange dots are evergreen species. Each point represents the mean value of a species. The black continuous line is the correlation considering species altogether. All regressions are highly significant ($P < 0.001$).

1030



1031

1032 **Fig. 9.** Main relationships between photosynthesis net rate ($A_{N.leaf}$) and phloem anatomical
1033 traits (cross-sectional area (A_p) and hydraulic diameter (d_{hp})). Deciduous as blue triangles
1034 and evergreen species as orange dots. Each point represents the mean value of a species.
1035 The black continuous line is the correlation considering species altogether. Both
1036 regressions are highly significant ($P < 0.001$).