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Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species

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Abstract

Stomatal conductance (g_s) and mesophyll conductance (g_m) represent major constraints to photosynthetic rate (A), and these traits are expected to coordinate with leaf hydraulic conductance (K_{leaf}) across species, under both steady-state and dynamic conditions. However, empirical information about their coordination is scarce. In this study, K_{leaf}, gas exchange, stomatal kinetics, and leaf anatomy in 10 species including ferns, gymnosperms, and angiosperms were investigated to elucidate the correlation of H_2O and CO_2 diffusion inside leaves under varying light conditions. Gas exchange, K_{leaf}, and anatomical traits varied widely across species. Under light-saturated conditions, the A, g_s, g_m, and K_{leaf} were strongly correlated across species. However, the response patterns of A, g_s, g_m, and K_{leaf} to varying light intensities were highly species dependent. Moreover, stomatal opening upon light exposure of dark-adapted leaves in the studied ferns and gymnosperms was generally faster than in the angiosperms; however, stomatal closing in light-adapted leaves after darkening was faster in angiosperms. The present results show that there is a large variability in the coordination of leaf hydraulic and gas exchange parameters across terrestrial plant species, as well as in their responses to changing light.

KEYWORDS

leaf anatomy, leaf hydraulic conductance, light, mesophyll conductance, photosynthesis, plant evolution, stomatal conductance

1 | INTRODUCTION

In terrestrial plants under saturating light, photosynthesis rates are limited by stomatal and mesophyll diffusion conductances (g_s and g_m, respectively) and by biochemical limitations (related to either carboxylation velocity, V_{cmax}, or photochemical and Calvin cycle activities setting a maximum electron transport rate, J_{max}). Grassi and Magnani (2005) established a method to estimate the partial contribution of each limiting factor to total photosynthetic limitation. In most angiosperms under non-stress conditions, stomatal (l_s), mesophyll (l_m), and biochemical (l_b) limitations often co-limit photosynthesis to a similar extent, although in some cases, l_b exerts a greater influence (Carriqui et al., 2015; Tomas et al., 2013). Contrarily, in gymnosperms (Veromann-Jurgenson, Tosens, Laanisto, & Niinemets, 2017) and ferns (Carriqui et al., 2015; Tosens et al., 2015), l_s and l_m generally constrain photosynthesis to a greater extent than l_b. This is related to the facts that, in contrast to angiosperms, ferns and gymnosperms tend to have larger stomata but in fewer numbers, which leads to lower g_s (de Boer et al., 2016; Franks & Beerling, 2009; Jordan, Carpenter, Koutoulis, Price, & Brodribb, 2015), and thicker cell walls and fewer chloroplasts, which leads to lower g_m (Carriqui et al., 2015; Tosens et al., 2015; Veromann-Jurgenson et al., 2017). In addition to these generalities, there are clear differences among species in the factors that limit photosynthesis the most (Carriqui et al., 2015; Tomas et al., 2013; Tosens et al., 2015; Veromann-Jurgenson et al., 2017).

The maximum stomatal conductance (and hence l_s) of a given species is related both to its stomatal density and size (de Boer et al., 2016; Franks & Beerling, 2009; Jordan et al., 2015) and to leaf hydraulic conductance (K_{leaf}; Brodribb & Holbrook, 2004; Brodribb, Feild, & Jordan, 2007; Scoffoni et al., 2016; Xiong et al., 2015). K_{leaf} represents the efficiency of water transport through leaves; water enters the leaf vein system from the petiole and then is transported through the bundle sheath and mesophyll cells before evaporating and diffusing out of stomata. K_{leaf} consists of two components: the inside- (K_x) and outside-

xylem (K_{ox}) conductances. Previous studies have shown a large variability between species in the proportion of hydraulic resistance distributed inside the xylem versus outside the xylem (Nardini, Gortan, & Salleo, 2005; Sack, Tyree, & Holbrook, 2005; Scoffoni et al., 2016; Scoffoni & Sack, 2015). K_x is mainly constrained by leaf vein density (VLA) and anatomy, whereas K_{ox} is related to VLA, and the anatomical and biochemical properties of the mesophyll and bundle sheath (Buckley, John, Scoffoni, & Sack, 2015; Caringella, Bongers, & Sack, 2015; Scoffoni et al., 2015). On this basis, some studies have suggested that VLA is one of the major driver of K_{leaf} variation across species (Brodribb & Feild, 2010; Sack & Scoffoni, 2013).

On the other hand, the maximum mesophyll conductance (and hence l_m) seems mostly related to cellular anatomical features, most notably cell wall thickness and chloroplast disposition in cells (Carriqui et al., 2015; Tosens et al., 2015; Veromann-Jurgenson et al., 2017). In addition, a substantial degree of coordination between g_s and g_m across species has been shown (Flexas et al., 2013). Due to this coordination and to the fact that CO_2 and H_2O may move partially along shared pathways within mesophyll tissues, some correlation between K_{leaf} and g_m across species under steady-state conditions should be expected (Flexas, Scoffoni, Gago, & Sack, 2013; Xiong, Flexas, Yu, Peng, & Huang, 2017). However, the coordination of g_s, g_m, and K_{leaf} across the terrestrial plant phylogeny has been little studied. Therefore, the first aim of this study is to investigate the coordination of photosynthetic gas exchanges and K_{leaf} in fern, gymnosperm, and angiosperm species.

In addition to steady-state conditions, the responses of g_s, g_m, and K_{leaf} to environmental changes have attracted increasing attention because their dynamics can influence plant performance including water use efficiency (WUE) over the course of a day (Flexas et al., 2013; Guyot, Scoffoni, & Sack, 2012; Lawson & Blatt, 2014; Lawson, Kramer, & Raines, 2012; Scoffoni, Pou, Aasamaa, & Sack, 2008). The response of g_s to dynamic light is expected to maintain high WUE, although the regulation mechanisms are still debated (Brodribb & McAdam, 2011; Franks, 2013; Franks & Britton-Harper, 2016; McAdam & Brodribb, 2012b). Recently, a series of studies (Brodribb & McAdam, 2011; McAdam & Brodribb, 2012a; McAdam & Brodribb, 2012b; McAdam & Brodribb, 2013; McAdam & Brodribb, 2015) claimed that stomatal movements in vascular plants are regulated by two types of processes: passive and active. According to this hypothesis, g_s regulation in ferns would be mostly passive, as it is mainly related to positive leaf water potential regulation and is sensitive to darkness but insensitive to elevated CO_2 and abscisic acid concentrations. In contrast, for gymnosperms and angiosperms, the g_s response to environmental changes is more active and related to multiple metabolic pathways, especially abscisic acid signalling, light, and CO_2 internal concentration sensing (Shimazaki, Doi, Assmann, & Kinoshita, 2007). The g_s response to darkness in ferns may be related to red-light receptors (Doi, Kitagawa, & Shimazaki, 2015; Doi & Shimazaki, 2008). Nevertheless, Franks and Britton-Harper (2016) recently found that the g_s of three ferns also responded to CO_2 concentration, though this response was delayed in time compared to angiosperms; this result suggests a common stomatal regulation pathway among vascular plants. In turn, the g_m response to light changes has been estimated in several angiosperms, and the results have shown that g_m generally responds to different light intensities (Douthe, Dreyer, Epron, & Warren, 2011; Flexas et al., 2007; Xiong et al., 2015), though not in some species (Tazoe, von Caemmerer, Badger, & Evans, 2009). Moreover, it is important to note that some simulation studies indicated that the response of g_m to light may be artefactual due to methodological pitfalls (Gu & Sun, 2014; Tholen, Ethier, Genty, Pepin, & Zhu, 2012). Similar to g_m, the response of K_{leaf} to light changes is highly dependent on species (Cochard et al., 2007; Rockwell, Holbrook, & Zwieniecki, 2011; Scoffoni et al., 2008). However, how the coordinated response of g_s, g_m, and K_{leaf} to light intensities varies in different terrestrial plants is still unclear.

Some coordination of g_s, g_m, and K_{leaf} under dynamic environmental conditions is expected. Many studies have observed that g_s and g_m responded in parallel to irradiance, CO_2, temperature, and drought stress (Flexas et al., 2007; Flexas, Ribas-Carbo, Diaz-Espejo, Galmes, & Medrano, 2008; Xiong et al., 2015). In contrast, Warren (2008) found that g_m was insensitive to vapour pressure deficit (VPD) whereas g_s was sensitive. Flexas et al. (2013) proposed that the g_s–g_m relationship under dynamic conditions may be mediated by K_{leaf}, because CO_2 likely shares a common pathway with H_2O inside leaves, and liquid water flux should match with vapour flux (Flexas et al., 2013; Xiong et al., 2017). Still, Guyot et al. (2012) investigated the responses of g_s and K_{leaf} to both leaf dehydration and two light intensities in four angiosperm species and found that the g_s response to light and drought was somewhat independent of the response of K_{leaf}. Moreover, Martins, McAdam, Deans, DaMatta, and Brodribb (2016) found that the response of g_s to VPD was limited by K_{leaf} in gymnosperms and ferns. These results indicated that the correlation of g_s and K_{leaf} under dynamic conditions may be species dependent. Therefore, the second aim of this study is to investigate how the coordination of the responses of g_s, g_m, and K_{leaf} to light intensity varies across terrestrial plants.

In addition to the direction and extent of changes, the speeds of g_s and A in response to light changes are also thought to impact WUE. Species with rapid g_s response to light and darkness tend to have a high WUE (Lawson & Blatt, 2014). Recently, many studies have focused on the speed of stomatal closing and opening in response to light changes, which are termed stomatal kinetics, and suggested that stomatal kinetics were related to stomatal size (Elliott-Kingston et al., 2016; Lawson & Blatt, 2014; Martins et al., 2016; McAusland et al., 2016). However, the stomatal kinetic response to light changes was typically investigated in angiosperms and was seldom studied in ferns and gymnosperms. Moreover, the effects of leaf anatomical traits and K_{leaf} on stomatal kinetics in response to light are unclear. Hence, the third aim of this study is to estimate the species variation in stomatal kinetic response to light as well as its correlation with leaf anatomical and hydraulic traits.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Two ferns (F), two gymnosperms (G), and six angiosperms (A) of different functional groups and growth habits were evaluated in this study (Table 1). Young plants of *Phlebodium aureum* (F), *Nephrolepis cordifolia* (F), *Taxus baccata* (G), *Ginkgo biloba* (G), and *Centella asiatica* (A) and

TABLE 1 Leaf anatomical/morphological traits for the 10 studied species

Species	Family	Habitat	LMA (g/m^2)	VLA_{major} (mm/mm^2)	VLA_{minor} (mm/mm^2)	VLA (mm/mm^2)	SD (mm^{-2})	SS (μm^2)
Ferns								
Phlebodium aureum	Polypodiaceae	Tropical rainforests	89.5 ± 10.7c	0.08 ± 0.01g	1.39 ± 0.14f	1.47 ± 0.13g	57.0 ± 4.5ef	3674 ± 433b
Nephrolepis cordifolia	Lomariopsidaceae	Tropical rainforests	31.9 ± 3.9f	0.12 ± 0.01g	1.19 ± 0.09f	1.31 ± 0.04g	27.2 ± 2.8f	4961 ± 694a
Gymnosperms								
Taxus baccata	Taxaceae	Terrestrial/wood	110.4 ± 4.9b	0.48 ± 0.02ef	—	0.48 ± 0.02h	101.0 ± 8.0e	2559 ± 318c
Ginkgo biloba	Ginkgoaceae	Terrestrial/wood	90.3 ± 6.3c	1.78 ± 0.12a	—	1.78 ± 0.12g	65.1 ± 5.8ef	2490 ± 413c
Angiosperms								
Nerium oleander	Apocynaceae	Terrestrial/wood	148.8 ± 10.9a	0.54 ± 0.02de	7.40 ± 0.84d	7.94 ± 0.44d	—	—
Populus nigra	Salicaceae	Terrestrial/wood	64.3 ± 9.3de	0.67 ± 0.08cd	13.53 ± 1.26a	14.21 ± 0.89a	226.3 ± 32.4c	1447 ± 242d
Gossypium hirsutum	Malvaceae	Terrestrial/herb	76.5 ± 4.6cd	0.68 ± 0.02cd	9.56 ± 1.05c	10.25 ± 0.77c	269.8 ± 89.6c	1285 ± 198d
Helianthus annuus	Asteraceae	Terrestrial/herb	58.4 ± 0.9e	0.95 ± 0.06b	11.41 ± 1.48b	12.36 ± 0.76b	575.8 ± 32.6a	1152 ± 297d
Centella asiatica	Apiaceae	Wet/herb	39.7 ± 4.4f	0.36 ± 0.04f	3.78 ± 0.53e	4.41 ± 0.14f	163.3 ± 20.3d	1248 ± 216d
Oryza sativa	Poaceae	Wet/grass	44.0 ± 3.3f	0.71 ± 0.06c	4.84 ± 0.39e	5.55 ± 0.25e	350.0 ± 63.9b	346 ± 45e

Note. LMA = leaf mass per area; VLA_{major} = major vein length per area; VLA_{minor} = minor vein length per area; VLA = leaf vein length per area; SD = stomatal density; SS = stomatal size. Different letters indicate significant differences ($p \leq .05$; $n = 3$–6).

seeds of *Gossypium hirsutum* (A), *Helianthus annuus* (A), and *Oryza sativa* (*cv*. Shanyou 63; A) were obtained from commercial suppliers. Shoot cuttings of *Populus nigra* (A) and *Nerium oleander* (A) were collected from single trees of each species in the campus of Illes Balears University, Palma, Spain (climate information can be found in http://plantmed.uib.es/Ingles/INTRANET.html).

P. aureum, *N. cordifolia*, and *C. asiatica* plants were transplanted to 3.0 l pots, and *T. baccata* and *G. biloba* plants were transplanted to 10.0 l and 30.0 l pots, respectively, in the summer of 2015. The medium in all the pots was a mixture of perlite and horticultural substrate (1:3), and the plants were grown in natural conditions at the campus of Illes Balears University. The shoot cuttings of *P. nigra* and *N. oleander* were transplanted to 10.0 l pots containing a mixture of perlite and horticultural substrate (1:3) when roots emerged in 2015. *G. hirsutum* and *H. annuus* seeds were directly seeded in the experimental field on the campus of Illes Balears University. The plants were irrigated daily using a drip irrigation system. The seeds of *O. sativa* were directly seeded in 3.0 l pots containing a mixture of perlite and horticultural substrate (1:3) in a growth chamber (12-hr photoperiod, 350 μmol quanta m^{-2} s^{-1} light intensity, 25 °C/20 °C day/night temperature, and relative humidity ~50%). Plants were well watered at all times and for the wet habitat species *C. asiatica* at least a 2-cm water layer was maintained in the pots for the duration of growth. All measurements were performed between June and August of 2016.

2.2 | Gas exchange

An open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) with an integrated fluorescence leaf chamber (LI-6400-40, LI-COR) was used to simultaneously measure leaf gas exchange and chlorophyll fluorescence. For each leaf, a light response curve was measured using five light intensities; in order, these are 2,000, 1,500, 1,000, 500, and 0 μmol·m^{-2}·s^{-1} (10:90% blue:red light). During the measurements, the reference CO_2 concentration was adjusted to 400 μmol/mol with a CO_2 mixture. Block temperature was set at 25 °C, and the leaf-to-air VPD was maintained between 1.5 and 2.0 kPa. The flow rate was set to 300 μmol/s when the photosynthetic rate was higher than 5 μmol·m^{-2}·s^{-1}, and to 150 μmol/s when the photosynthetic rate was lower than 5 μmol·m^{-2}·s^{-1}. After the leaf reached a steady state (a fluctuation of g_s less than 0.05 mol·m^{-2}·s^{-1} during a 10-min period), usually after 30 to 120 min, gas exchange parameters, steady-state fluorescence (F_s), and maximum fluorescence (F_m`) were recorded. Under 0 μmol·m^{-2}·s^{-1} photosynthetically active radiation (PAR), only the gas exchange parameters were recorded.

The actual photochemical efficiency of Photosystem II (Φ_{PSII}) was calculated as follows:

$$\Phi_{PSII} = \frac{\left(F_m^{`} - F_s\right)}{F_m^{`}}.$$

The electron transport rate (J) was then calculated as follows:

$$J = \Phi_{PSII} \cdot PAR \cdot \alpha\beta,$$

where α is the leaf absorbance and β is the partitioning of absorbed quanta between Photosystems II and I. The product $\alpha\beta$ was estimated

from the slope of the relationship between Φ_{PSII} and $4\Phi_{CO_2}$ (i.e., the quantum efficiency of gross CO_2 fixation), which was obtained by measuring the photosynthetic light response curves under non-photorespiration conditions (i.e., O_2 < 1%).

The variable *J* method (Harley, Loreto, Di Marco, & Sharkey, 1992) was used to calculate g_m and C_c. C_c and g_m were calculated as follows:

$$C_c = \frac{\Gamma^*(J + 8(A + R_d))}{J - 4(A + R_d)},$$

$$g_m = \frac{A}{C_i - C_c},$$

where Γ^* represents the CO_2 compensation point in the absence of respiration; a typical value, 40 μmol/mol, was used in this study. For each data point generated, we checked whether it met the criterion ($10 > dC_c/dA > 50$; Harley et al., 1992). In the current study, the day respiration (R_d) was calculated as 1/2 of the dark respiration.

2.3 | Quantitative analysis of photosynthetic limitations

Relative photosynthetic limitations including stomatal (l_s), mesophyll (l_s), and biochemical (l_b) relative limitations were calculated for all species according to Grassi and Magnani (2005).

$$l_s = \frac{g_t/g_s \cdot \partial A/C_c}{g_t + \partial A/\partial C_c},$$

$$l_m = \frac{g_t/g_m \cdot \partial A/\partial C_c}{g_t + \partial A/\partial C_c},$$

$$l_b = \frac{g_t}{g_t + \partial A/\partial C_c},$$

where g_t is the total CO_2 diffusion conductance ($g_t = 1/(1/g_s + 1/g_m)$) and $\partial A/\partial C_C$ is the slope of the *A* versus C_C response curve. According to the Farquhar model (Farquhar, von Caemmerer, & Berry, 1980), $\partial A/\partial C_C$ can be calculated as follows:

$$\partial A/\partial C_c = V_{c\,max} \frac{\Gamma^* + K_c(1 + O/K_o)}{(C_c + K_c(1 + O/K_o))^2},$$

where V_{cmax} is the maximum velocity of carboxylation, K_c and K_o are the Rubisco Michaelis–Menten constants for CO_2 and oxygen, respectively, and O is the oxygen concentration in chloroplast. In the current study, we used the chlorophyll fluorescence-derived value *J* instead of V_{cmax} to calculate relative photosynthetic limitations (Carriqui et al., 2015; Galle et al., 2009; Galle, Florez-Sarasa, Aououad, & Flexas, 2011).

2.4 | Stomatal kinetics

We estimated the changes in stomatal conductance following step changes in light intensity (PAR) between 0 and 1,500 μmol·m^{-2}·s^{-1} using the Li-COR 6400. The day before measuring, plants were moved to the lab and covered with black bags, and the target leaves of *G. hirsutum* and *H. annuus* under field conditions were covered with silver paper. To ensure full hydration during the measurements, the plants were irrigated with 1,000 to 2,000 ml deionized water before measurements were performed. The darkness-acclimated leaves were first equilibrated at a PAR of 0 μmol·m^{-2}·s^{-1} until both *A* and g_s reached steady state (<3% change in rate during a 10-min period). Then, the PAR was increased to 1,500 μmol·m^{-2}·s^{-1} until a steady state was reached, before returning to 0 μmol·m^{-2}·s^{-1}. Data were logged every 30 s. The CO_2 concentration and block temperature in the cuvette were set to 400 μmol/mol and 25 °C, respectively. The VPD was maintained between 1.5 and 2.0 kPa.

2.5 | Leaf hydraulic conductance

Before measuring, the fully watered potted plants were brought into the lab, covered with black bags and rehydrated overnight. For the field plants, branches with at least five leaves were collected and recut under ultrapure water. The branches were covered in black plastic bags and rehydrated overnight. K_{leaf} was measured using the evaporative flux method (Sack & Scoffoni, 2012; Scoffoni et al., 2015; Xiong et al., 2015) on non-senescent and fully expanded leaves. To ensure a tight seal with the tubing that supplied water, the petioles were wrapped with thread seal tape (polytetrafluoroethylene film). For the *O. sativa* leaves, first, the leaf sheath coated to a cone-shaped plastic stick, and then the outside of the sheath was wrapped in thread seal tape (Xiong et al., 2017). The tubing system was connected to a plastic Erlenmeyer flask (250 ml) with degassed solution situated on an analytical balance (ABT 320-4M, KERN, Balingen, Germany). Before measuring, leakage was assessed checked by creating a high gradient (~60 cm) between the leaves and the water surface in the Erlenmeyer flask. To estimate the light response of K_{leaf}, a light-emitting diode with blue and red light (APO4, Eiviled 2010, Illes Balears, Spain) was used, and the light intensities (2,000, 1,500, 1,000, and 500 μmol·m^{-2}·s^{-1} and darkness) on the leaf surface were controlled by adjusting distances between the leaf and the light source. The light intensity was measured with a quantum sensor (Li-190R, LI-COR). The "darkness" in the current study was defined as the ambient light intensity (<10 μmol·m^{-2}·s^{-1}) in the lab. Leaf temperature was controlled between 23 to 27 °C using an air conditioner. When leaves reached a steady state (the water weight lost linearly with time, typically ~50–240 min, highly dependent on species), the weight of the water was recorded every 60 s, and the water flow rate was calculated as the slope of the linear regression between weight and time. The leaf area was measured using ImageJ (https://imagej.nih.gov/ij/), and then the liquid water flow rate was normalized by leaf area (*E*). The leaves were equilibrated in bags for 30 min before the leaf water potential was measured with a pressure chamber (Model 3000, Soilmoisture Equipment Corp., Santa Barbara, CA, USA). K_{leaf} was calculated as follows:

$$K_{leaf} = \frac{E}{\Psi_{water} - \Psi_{leaf}},$$

where the Ψ_{water} is the water potential of distilled water (=0 MPa).

2.6 | Leaf vein density

To determine vein traits, one leaf from each of three individuals per species was chemically cleared in 15% NaOH (w/v) and bleach following a standard protocol (Scoffoni et al., 2015). The cleared leaves were stained with safranin and fast green. Leaves were scanned for quantification of leaf area and vein length. In the present study, the 1° vein length of the ferns, 1° and 2° vein lengths of *O. sativa*, and 1°, 2°, and 3° vein lengths of the dicotyledonous species were measured based on the entire leaf images. To measure the minor vein lengths of ferns and angiosperms as well as the vein length of gymnosperms, a light microscope (U-TVO.5XC; Olympus, Tokyo, Japan) with a 5× objective and digital camera were used, and pictures were taken at the top, middle, and bottom of each leaf. Leaf area and vein length were manually measured using ImageJ. In the current study, the major vein is defined as the 1° vein in the ferns and gymnosperms, the sum of the 1° and 2° veins in *O. sativa*, and the sum of the 1°, 2°, and 3° veins in dicotyledon leaves. The 2° veins in ferns, 3° veins in *O. sativa*, and the veins of any order higher than 3° in dicotyledon leaves were considered as minor veins.

2.7 | Stomatal traits

Six small leaf discs (approximately 10 × 10 mm) from the central portion of each leaf (three leaves from three plants per species) were collected; however, only four small leaf discs per leaf were collected for *T. baccata* leaves due to its extremely small leaves. Leaf discs were cleared with 10% NaOH (w/v) hydrotrope solution for 24 hr, followed by an overnight treatment in 50% ethanol solution. If necessary, leaf discs were bleached in 10% H_2O_2 to remove background coloration. A 5% solution of safranin (in ethanol) was used to stain the leaves. Images of both the abaxial and adaxial sides were taken using a light microscope (U-TVO.5XC; Olympus, Tokyo, Japan). Stomatal density (SD, mm^{-2}), guard cell length (GL), width of the entire stoma at the centre (SW), stomatal pore length (PL), pore width at centre of the stoma (PW), and guard cell width at the centre of the stoma (GW) were manually recorded using ImageJ. In the current study, stoma size (SS, μm^2) was defined as an ellipse with its major axis equal to GL and its minor axis equal to SW, and maximum stomatal pore area (α_{max}, μm^2) was defined as an ellipse with its major axis equal to PL and its minor axis equal to PW. Because the stomata of *N. oleander* are located in depressions of the leaf surface (Figure 1), this species was excluded from the stomatal feature analysis.

Maximum theoretical stomatal conductance to CO_2 as defined by stomatal anatomy (g_{s_max}, $mol{\cdot}m^{-2}{\cdot}s^{-1}$) was estimated for each species using a double-end-correction version of the equation (Dow & Bergmann, 2014; Franks & Farquhar, 2001; Xiong et al., 2017) by

$$g_{s\_max} = \frac{d \cdot SD \cdot a_{max}}{1.6v\left(PD + \frac{\pi}{2}\sqrt{\frac{a_{max}}{2}}\right)},$$

where d is the diffusivity of water in air (24.9×10^{-6} m^2/s, at 25 °C), v is the molar volume of air (22.4×10^{-3} m^3/mol, at 25 °C and 101.3 kPa), PD is the stomatal pore depth, which is equal to GW in the current study, and π is the mathematical constant. The g_{s_max} for each leaf was calculated as the sum of g_{s_max} abaxial and adaxial.

2.8 | Statistical analysis

One-way ANOVA analysis was used to test for differences in measured traits (in Table 1) among species. Regression analyses were performed with mean values to test the correlations between parameters. Regressions were fitted by linear models, except the regression between stomatal density and size, which was fitted by a power model ($y = ax^b$). Regression lines were shown for $p < .05$. All analyses were performed in R version 3.3.1 (https://cran.r-project.org).

3 | RESULTS

3.1 | Variation of gas exchange, leaf hydraulic conductance, and anatomy across species

Leaf anatomical, photosynthetic, and hydraulic traits varied substantially across the species selected for this study. Leaf shapes, number of vein orders, and vein arrangement showed considerable variation among species (Figure 1; Table 1), and leaf mass per area (LMA), VLA_{major}, VLA_{minor}, and VLA varied from 4.7-fold (LMA) to 29.6-fold (VLA) across species (Table 1). The species also varied significantly in stomatal density and size (Table 1 and Figure 1). Although the photosynthetic rates of ferns saturate at much less than 2,000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, those of angiosperms saturate much closer to 2,000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ (Figure S1). Hence, a PAR of 2,000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ was considered as saturating light in order to compare all the species. Across all selected species, a 22.1-fold range of variation was found for saturated photosynthetic rate (A_{sat}), 38.2-fold for light-saturated g_s (g_{s_sat}), 19.8-fold for light-saturated g_m (g_{m_sat}), and 10.0-fold for light-saturated K_{leaf} (K_{leaf_sat}; Figure 2).

3.2 | Photosynthetic limitations

The quantitative limitation analysis (Figure 3) shows that, on average, A in the studied angiosperms was mainly limited by biochemical factors (l_b, 0.58), whereas the limitations of stomatal conductance (l_s, 0.21) and mesophyll conductance (l_m, 0.21) were at the same level. Across the angiosperms, l_b ranged from 0.47 to 0.71, whereas l_s and l_m ranged from 0.13 to 0.30, and from 0.13 to 0.26, respectively (Figure 3b). In contrast, in the studied ferns, the stomatal conductance (l_s, 0.49) and mesophyll conductance (l_m, 0.30) were the two most important factors in limiting A, whereas the biochemical (l_b, 0.21) limitations were less important. In the gymnosperms, A was mostly limited by mesophyll conductance (l_m, 0.39), followed by stomatal conductance (l_s, 0.33) and biochemical factors (l_b, 0.28).

3.3 | Correlation between leaf hydraulic conductance and gas exchange

Across the selected species, as expected, strong pairwise correlations were observed between K_{leaf_sat} and either A_{sat} ($r^2 = .87$; $p < .001$), g_{s_sat} ($r^2 = .73$; $p = .002$), or g_{m_sat} ($r^2 = .65$; $p = .005$; Table 2;

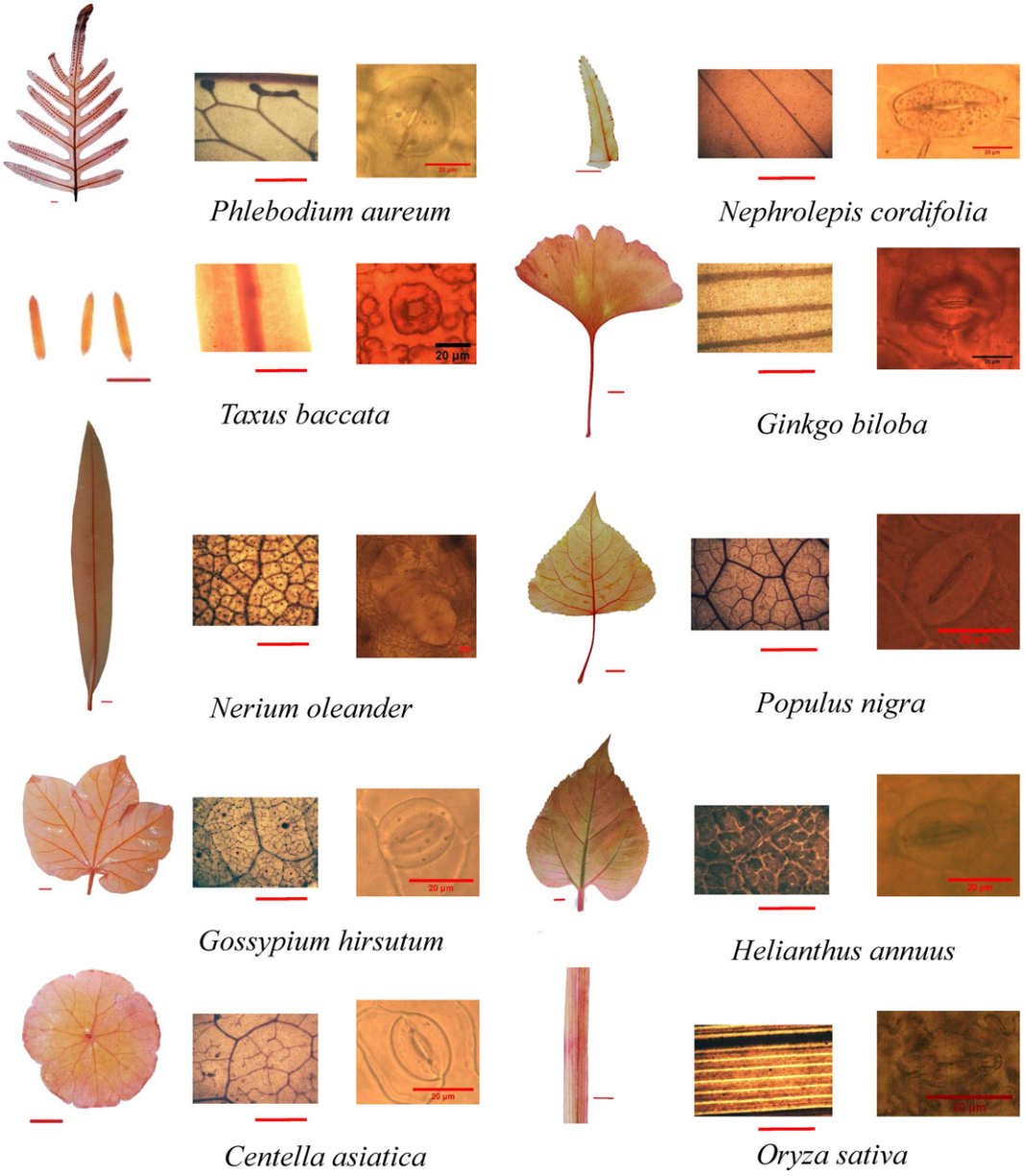

FIGURE 1 Representative anatomical sections showing the diversity in leaf size, shape, venation architecture, and stomatal appearance across the 10 studied species. For each species, the first, second, and third columns represent views of whole leaves, epidermal surfaces, and individual stomata, respectively. The scale bars represent 1 cm, 1 mm, and 20 μm, respectively. Note that in the stomata image of *N. oleander*, stomata are not seen due to the presence of stomatal crypts in this species

Figure 4a–c). A_{sat} was also positively correlated with g_{s_sat} (r^2 = .78; p < .001) and g_{m_sat} (r^2 = .83; p < .001) across species (Table 2; Figure S2). Moreover, tight correlations among A, K_{leaf}, g_s, and g_m across light intensities and species were observed (Figure 4d–f). In the current study, we found no significant correlations between LMA and leaf physiological traits (Table 2), and the variation of LMA was independent of VLA variation across species. However, VLA was found to be correlated with both K_{leaf_sat} and A_{sat} (Table 2). Across the species, a negative correlation between stomatal density and stomatal size was observed (Figure 5). The anatomy-based maximum g_s (g_{s_max}) varied from 0.177 to 2.907 mol·m^{-2}·s^{-1}, and a tight correlation between g_{s_max} and g_{s_sat} (r^2 = .71; p = .005) was found (Figure S3). g_{s_sat} was also positively correlated with both stomatal density and stomatal size (Table 2; Figure 5), and stomatal density was a stronger predictor of g_{s_sat} than stomatal size (r^2 = .85 vs. .68; Figure 5).

3.4 | Dynamics of g_s, g_m, and K_{leaf} with light changes

In the current study, we found that the g_s of all species had a significant response to light changes, although the response patterns were species dependent (Figure S4). The g_s of two ferns and one angiosperm (the wet habitat species *C. asiatica*) only presented the light/dark shift response, but did not have any further response to varying light intensities from 500 to 2,000 μmol·m^{-2}·s^{-1}. Similar to g_s, the response patterns of g_m (Figure S5) and K_{leaf} (Figure S6) to light intensities were species dependent. The K_{leaf} of ferns, gymnosperms, and one angiosperm, again *C. asiatica*, showed no light response, whereas the rest

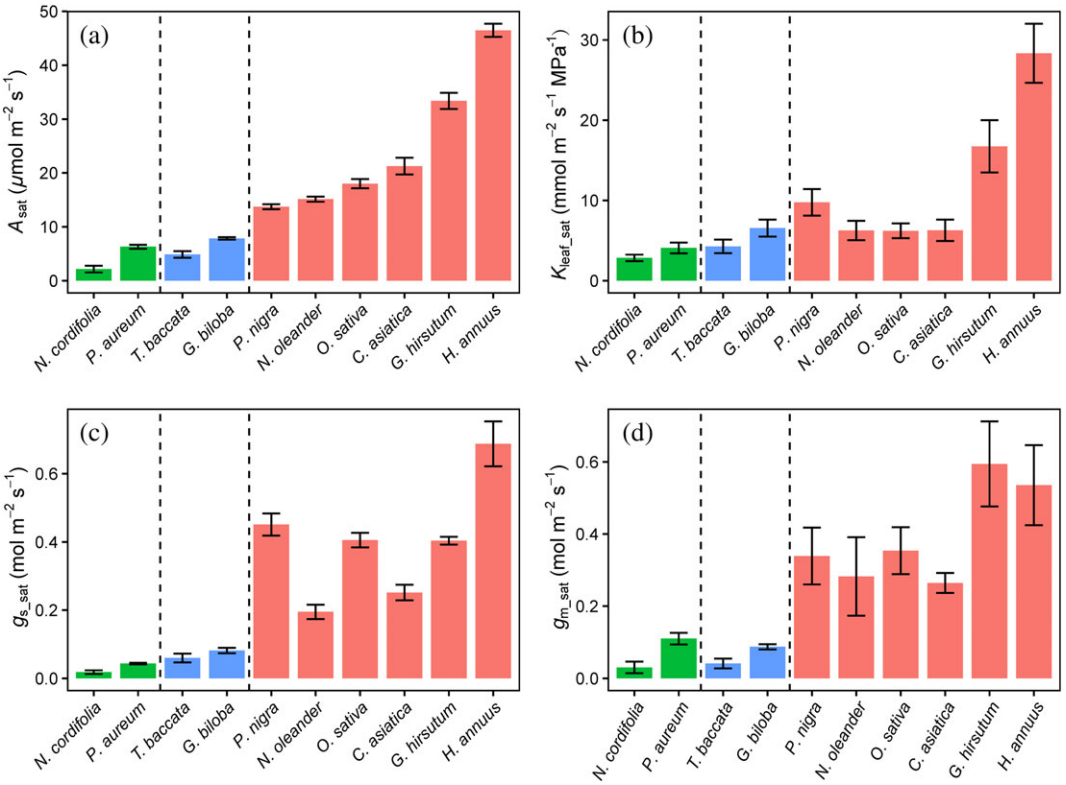

FIGURE 2 Light-saturated photosynthetic rate (A_{sat}), stomatal conductance (g_{s_sat}), mesophyll conductance (g_{m_sat}), and leaf hydraulic conductance (K_{leaf_sat}) for each species. All data were measured under 2,000 μmol·m^{-2}·s^{-1} PAR. Data are the means ± *SD* (n = 3–6). Green, blue, and red colors represent ferns, gymnosperms, and angiosperms, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

of the angiosperms responded to light intensity. There was a significant co-response of K_{leaf}, g_s, and g_m to light in some of the species, but not in others. Similar to g_{s_sat}, the steady-state g_s in darkness varied among species (Figure 6).

3.5 | Stomatal kinetics

The typical light-induced stomatal responses of 10 tested species are shown in Figure 7. From darkness to high light, the g_s of the studied ferns and gymnosperms increased faster than that of angiosperms; however, when transferred from high light to darkness, the stomata of angiosperms closed faster, except in the wet habitat species *C. asiatica* (Figures 6 and 7). Across the species, a weak positive relationship between the time required for a 50% increase of g_s under 1,500 μmol·m^{-2}·s^{-1} PAR (k_i) and VLA was observed (Table 2). Moreover, the time for a 50% decline of g_s under darkness (k_d) was positively correlated with stomatal size (but not k_i; Figure 8). The variations of k_i and k_d were independent of LMA and stomatal density across the species (Table 2).

4 | DISCUSSION

4.1 | Correlation between leaf hydraulic conductance and gas exchange in steady-state conditions

Our results show that, in general, the A_{sat} in the studied ferns and gymnosperms was lower than that in angiosperms (Figure 2). The low A_{sat} in ferns and gymnosperms was mostly related to the low CO_2 diffusion conductance (both g_{s_sat} and g_{m_sat}). This result is consistent with a previous study (Carriqui et al., 2015) in which gas exchange and leaf anatomical traits were compared in ferns and angiosperms only. The results from both Carriqui et al. (2015) and the current study indicate that photosynthetic capacity in ferns and gymnosperms is mainly constrained by CO_2 diffusion (see also Tosens et al., 2015; Veromann-Jurgenson et al., 2017), whereas in angiosperms, biochemical limitations have a larger role in constraining photosynthesis (Figure 3). Under a given condition, g_s is determined by the opening state of the stomata as well as stomatal characteristics including stomatal size and density. We found that, across species, g_{s_sat} increased with stomatal density but declined with stomatal size. In fact, the stomatal density of all the studied angiosperms is significantly higher than ferns and gymnosperms, but the stomata of angiosperms are significantly smaller. These results support the idea that, in the process of evolution, smaller stomata were selected to increase photosynthesis (Franks & Beerling, 2009). Under a given ambient condition, the opening status of stomata is related to the dynamic equilibrium between water supply and transpiration of the leaf, so the close link between K_{leaf_sat} and g_{s_sat} is not surprising (Brodribb et al., 2007; Brodribb & Holbrook, 2004; Scoffoni et al., 2015; Scoffoni et al., 2016). Similar to g_{s_sat}, a higher g_{m_sat} in angiosperms than in ferns as well as in gymnosperms was observed, and g_{m_sat} was correlated with K_{leaf_sat}. The g_{m_sat}–K_{leaf_sat} correlation may come from anatomical pathways shared in common between CO_2 diffusion and water transport though the mesophyll (Flexas et al., 2013; Xiong et al., 2017). Interestingly, the K_{leaf_sat} in the present study did not show a clear phylogenetic trend; although some angiosperm

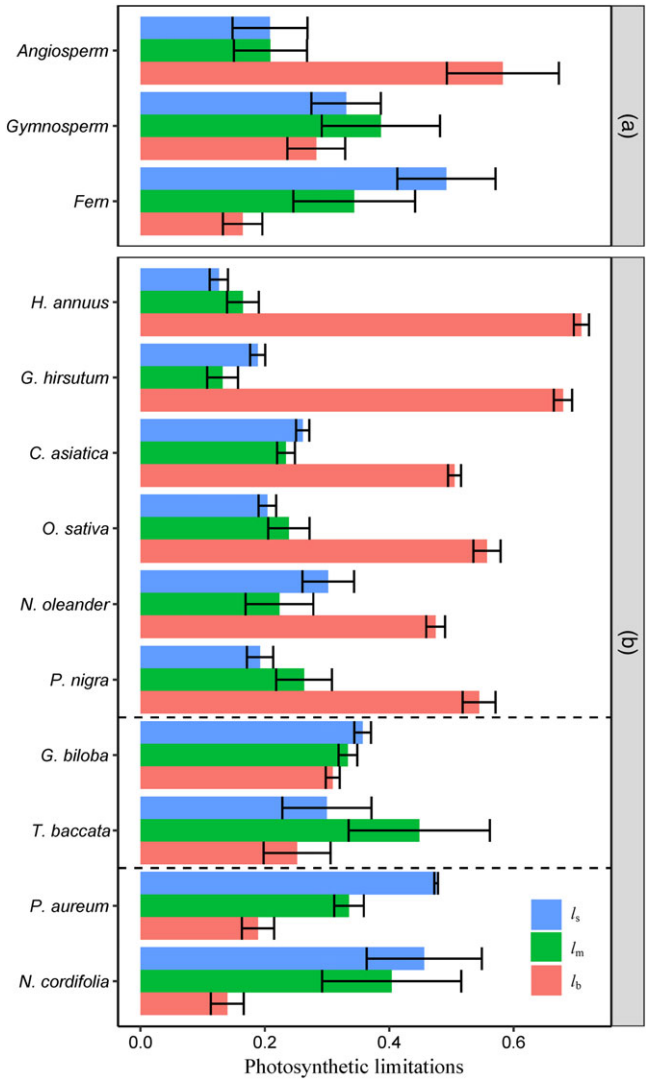

FIGURE 3 Quantitative limitation analysis averaged (a) for species within the three phylogenetic groups and (b) for each individual species. Total relative limitation (1.0) of photosynthesis is represented by the sum of stomatal conductance (l_s), mesophyll conductance (l_m), and biochemical (l_b) limitations. Data are the means ± *SD* (n = 3–6) [Colour figure can be viewed at wileyonlinelibrary.com]

species produced high K_{leaf_sat} associated with large VLA, others fell well within the same range as gymnosperms and ferns.

4.2 | Light responses of K_{leaf} and gas exchange

In general, K_{leaf} did not show a light response in the ferns and gymnosperms but did in the angiosperms, except for the wet habitat angiosperm *C. asiatica*. Several studies have reported a K_{leaf} light enhancement from darkness to a single light level (Cochard et al., 2007; Guyot et al., 2012; Nardini, Salleo, & Andri, 2005; Scoffoni et al., 2008), demonstrating the presence or absence of K_{leaf} variations with light. Nevertheless, full K_{leaf} light response curves have rarely been derived. Here, we first show that the light needed to saturate K_{leaf} varied widely across species, and the K_{leaf} in nine of 10 species was light saturated around 1000 μmol·m^{-2}·s^{-1}, a typical light intensity for K_{leaf} measurement with the evaporative flux method (Sack, Melcher, Zwieniecki, & Holbrook, 2002; Sack & Scoffoni, 2012). However, the saturated light intensity for K_{leaf} in *H. annuus* was higher than 1,000 μmol·m^{-2}·s^{-1}. Moreover, our result indicates that the rehydration kinetics method, in which K_{leaf} is typically measured under low irradiance (Blackman & Brodribb, 2011; Blackman, Brodribb, & Jordan, 2009), may potentially underestimate K_{leaf} for light sensitive species, and thus, the light intensity should be considered in future K_{leaf} estimations. The species-dependent behaviour of K_{leaf} response to light changes may be related to the outside-xylem compartment as suggested by previous studies (Cochard et al., 2007; Nardini et al., 2005; Scoffoni et al., 2008). On one hand, the light may regulate the expression of aquaporin genes in the bundle sheath and/or the mesophyll tissues, thus altering the K_{leaf} (Ben Baaziz et al., 2012; Lopez et al., 2013; Prado et al., 2013). On the other hand, changes in preferential water pathways could affect K_{leaf}, such as changes from apoplastic flow under low light to cell-to-cell flow under high light (Cochard et al., 2007). In the same way, the species-dependent K_{leaf} responses in our study could have been determined by different percentages of outside-xylem resistance, a trait which varies from 11 to 97% across species based on previous studies (Cochard, Nardini, & Coll, 2004; Sack et al., 2005; Sack, Streeter, & Holbrook, 2004; Scoffoni et al., 2016), or by some species-dependent light regulation of aquaporin expression and/or activity. Unfortunately, these two traits were not measured in the current study, meaning that only speculations can be made at this stage. Negative correlations between leaf water potential and the K_{leaf} and gas exchange traits have been reported by previous studies (Guyot et al., 2012; Scoffoni, McKown, Rawls, & Sack, 2012; Scoffoni, Rawls, McKown, Cochard, & Sack, 2011; Scoffoni & Sack, 2017). Here, we found that leaf water potential decreased with increasing light intensity in all species, although the decrease was smaller in ferns (Figure S7). Despite this fact, variations in K_{leaf} were not present in gymnosperms or ferns. This suggests that leaf water potential is not the only factor influencing gas exchange and K_{leaf} variation under changing light levels, reinforcing the idea that other potential factors (aquaporins among them) could be involved. In addition, the species that still showed K_{leaf} enhancement under high light intensity with a decreased leaf water potential could have potentially shown an even greater response to light if measured at high water potentials, and further detail investigations are needed to address this issue.

Light is a key stomatal opening signal. A positive correlation between g_s and light intensity has been observed and may originate in two processes. The first is a decrease in intercellular CO_2 concentration (C_i) as light increases due to an enhancement of A via activation of the ETR, because guard cells are regulated by turgor pressure and C_i (Franks & Britton-Harper, 2016; Lawson, Simkin, Kelly, & Granot, 2014; Mott, Sibbernsen, & Shope, 2008; Shimazaki et al., 2007). The second process is direct stomatal opening induced by blue-light sensors in guard cells (Doi et al., 2015). It could be important to note that g_s typically declines with leaf water potential decrease and, in this study, we observed decreasing leaf water potential at high light intensities in some of species. Hence, similar to K_{leaf}, the light response of g_s in some of species might be affected by leaf water potential, simultaneously. Several studies also observed an

TABLE 2 Correlation matrix between studied traits

	g_{s_sat}	g_{s_max}	g_{m_sat}	K_{leaf_sat}	LMA	VLA	SD	SS	k_i	k_d
A_{sat}	.78***	.76**	.83***	.87***	ns	.51*	.86***	ns	ns	ns
g_{s_sat}		.71**	.80***	.73**	ns	.72**	.75**	.59*	ns	.46*
g_{s_max}			.50*	.77**	ns	.49*	.91***	ns	ns	ns
g_{m_sat}				.65**	ns	.68**	.60*	.55**	.46*	.42*
K_{leaf_sat}					ns	.51*	.76**	ns	ns	ns
LMA						ns	ns	ns	ns	ns
VLA							.55*	ns	.41*	ns
SD								.71**	ns	ns
SS									ns	.62*
k_i										ns

Note. A_{sat} = saturated photosynthetic rate under 2,000 μmol·m^{-2}·s^{-1} PAR; g_{s_sat} = stomatal conductance under 2,000 μmol·m^{-2}·s^{-1} PAR; g_{m_sat} = mesophyll conductance under 2,000 μmol·m^{-2}·s^{-1} PAR; g_{s_max} = theoretical maximum stomatal conductance; K_{leaf_sat} = leaf hydraulic conductance under 2,000 μmol·m^{-2}·s^{-1} PAR; LMA = leaf mass per area; VLA = vein length per area; SD = stomatal density; SS = stomatal size; k_i = time for 50% increase of g_s under 1,500 μmol·m^{-2}·s^{-1} PAR; k_d = time for 50% decline of g_s under darkness.

*p < .05.

**p < .01.

***p < .001; ns = not significant.

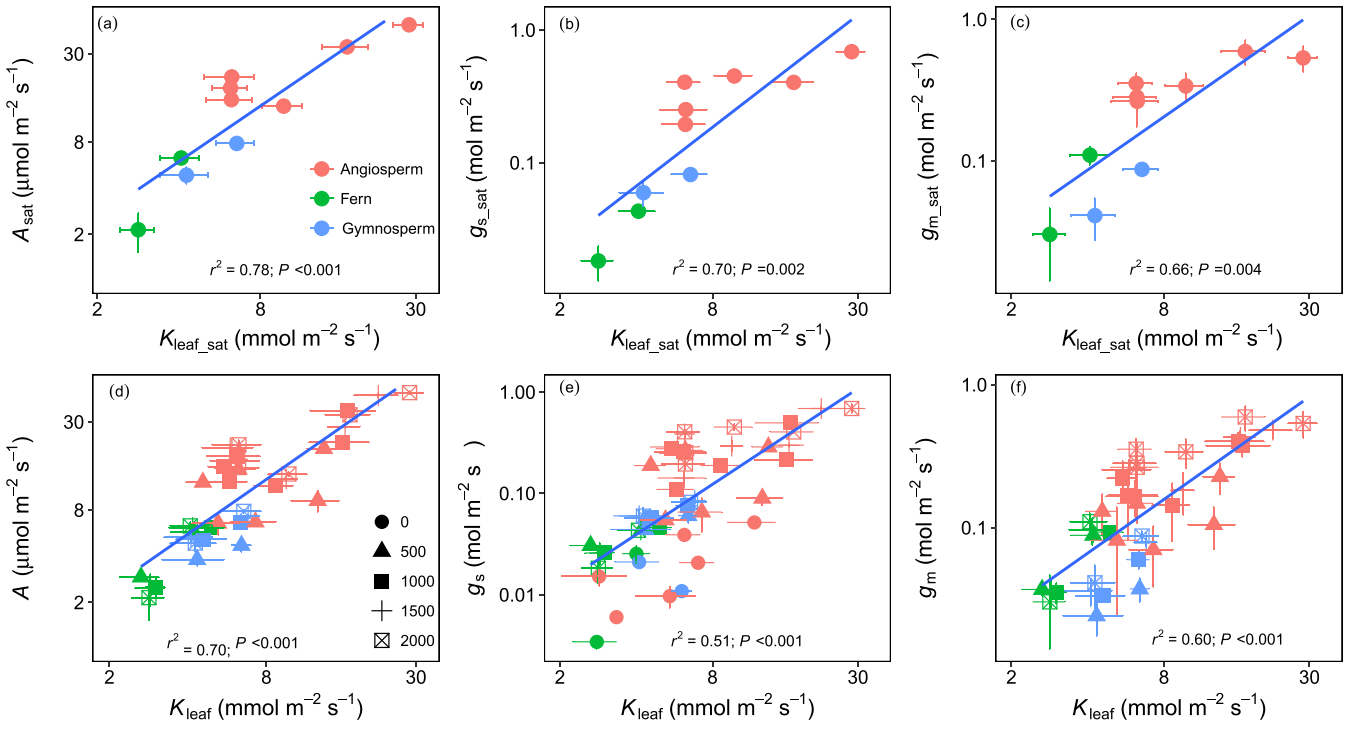

FIGURE 4 Correlations of (a,d) photosynthetic rate (A), (b,e) stomatal conductance (g_s), and (c,f) mesophyll conductance (g_m,) with leaf hydraulic conductance (K_{leaf}) across species (a–c) under light-saturated conditions and (d–f) across light intensities. Data are the means ± *SD* (n = 3–6). Green, blue, and red colors represent ferns, gymnosperms, and angiosperms, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

increasing g_m with increasing light intensity in several angiosperm species (Douthe et al., 2011; Flexas et al., 2007; Xiong et al., 2015). The response of g_m to light may be caused by (a) light regulation in aquaporin expression and/or activity at the plasma membrane and/or chloroplast membrane, (b) light-induced chloroplast movement (Flexas & Diaz-Espejo, 2014), or (c) other unknown factors. In the present study, we found that g_m responded to light in all angiosperms and gymnosperms. It should be acknowledged that, according to the simulations by Gu and Sun (2014), the response of g_m to light changes could be caused by methodological artefacts. However, in this case, all the species would be expected to have a similar response pattern, and thus, we consider the observed g_m responses to light to be valid at least for comparison purposes among species. In summary, in ferns K_{leaf}, g_s, and g_m show no response to light intensity, but stomata close in darkness. The studied gymnosperms presented no response for K_{leaf}, a partial response of g_m, and a complete response of g_s, and finally, all angiosperms except *C. asiatica* present a marked light response for all three parameters (Table 3). Interestingly, the wet

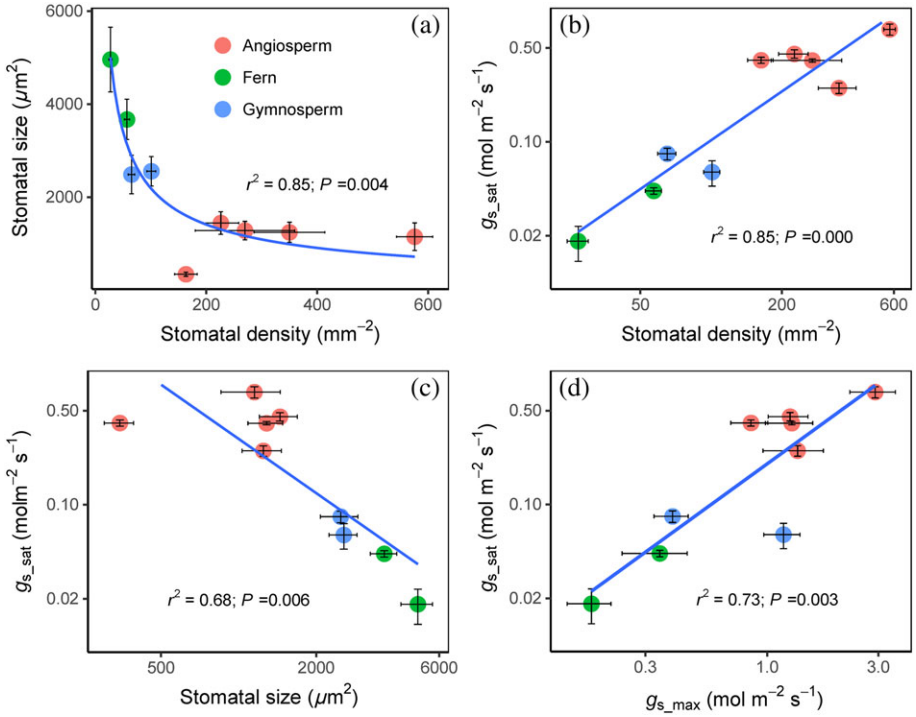

FIGURE 5 (a) Correlation between stomatal density and size across species, (b) correlation between stomatal density and light-saturated stomatal conductance (g_{s_sat}), (c) correlation between stomatal size and g_{s_sat}, and (d) correlation between theoretical maximum stomatal conductance (g_{s_max}) and g_{s_sat}. Data are the means ± *SD* (n = 3–6) [Colour figure can be viewed at wileyonlinelibrary.com]

habitat angiosperm *C. asiatica* lacks a response in all parameters, behaving similarly to the ferns analysed in the present study. We have observed similar lack of g_s response to light in several additional wet habitat angiosperms, but g_m and K_{leaf} were not evaluated (Douthe, unpublished results). Overall, on the basis of the observed patterns and recognizing that the number of species studied within each group is insufficient to be conclusive, it might be speculated that extant ferns may represent remnants of ancestral terrestrial vascular plants, with all the water- and gas-regulating attributes (K_{leaf}, g_s, and g_m) showing low steady-state values and no capacity of g_m and K_{leaf} to respond to light. Gymnosperms show a more advanced trait combination, still with low steady-state values but with some degree of response to light, whereas angiosperms represent the most evolved group, with higher steady-state values and a complete capacity of all traits to flexibly respond to light changes. Some angiosperms may have latter lost these capacities to adapt to particular environments, as illustrated here by the wet habitat species *C. asiatica*. Although merely speculative, this mechanistic evolutionary hypothesis agrees well with the humid and low-light habitat preferences of many ferns (Page, 2002), with the low relative evolutionary success of gymnosperms in terms of the current number of species (Bond, 1989), with the larger embolism risk in ferns compared to angiosperms (Brodribb, Bienaime, & Marmottant, 2016), and with the large evolutionary success and capacity to colonize a wide range of environments of angiosperms (Willis & McElwain, 2014). It is important to note that in the current study, we only investigated two ferns and two gymnosperms, which may not be representative of their entire lineages, but it would be interesting to increase species number, especially in basal groups, to check this point. These findings will support future works by revealing the evolution of the coordination of CO_2 diffusion and H_2O transport inside leaves.

4.3 | Stomatal kinetics

Under natural conditions, light is one of the most dynamic environmental factors. Therefore, in addition to the presence or absence of light responses, here, we estimated the stomatal kinetics in 10 species using a stepwise increase followed by a decrease of light intensity. We found that, in general, A and g_s were coupled during stomatal opening but uncoupled during stomatal closing. In all species, stomata closed when transferred to darkness and opened in full light, which supports previous studies that the blue-light response of stomatal opening is strongly conserved among species (Doi et al., 2015). In this study, we also observed that stomatal opening in ferns was faster than in angiosperms; meanwhile, fern stomatal closing was slower. Previous studies suggested that regulation of stomatal movements was different in ferns and angiosperms (Brodribb & McAdam, 2011; McAdam & Brodribb, 2012a; McAdam & Brodribb, 2012b). Stomatal movements in response to environmental changes in ferns are related to a passive mechanism that is mediated by leaf water potential, whereas in angiosperms, stomatal movements are mainly regulated by multiple metabolic interactions. Therefore, the different response speeds of stomata among species may be related to their different stomatal regulation mechanisms. When leaves adapt from light to darkness, the response of g_s in ferns is slow because the leaf water potential is maintained or even increased by the decrease in transpiration rate (Figure S7). However, angiosperms leaves may possess some light-dependent *de novo* synthesis reactions and/or pathways related to stomatal

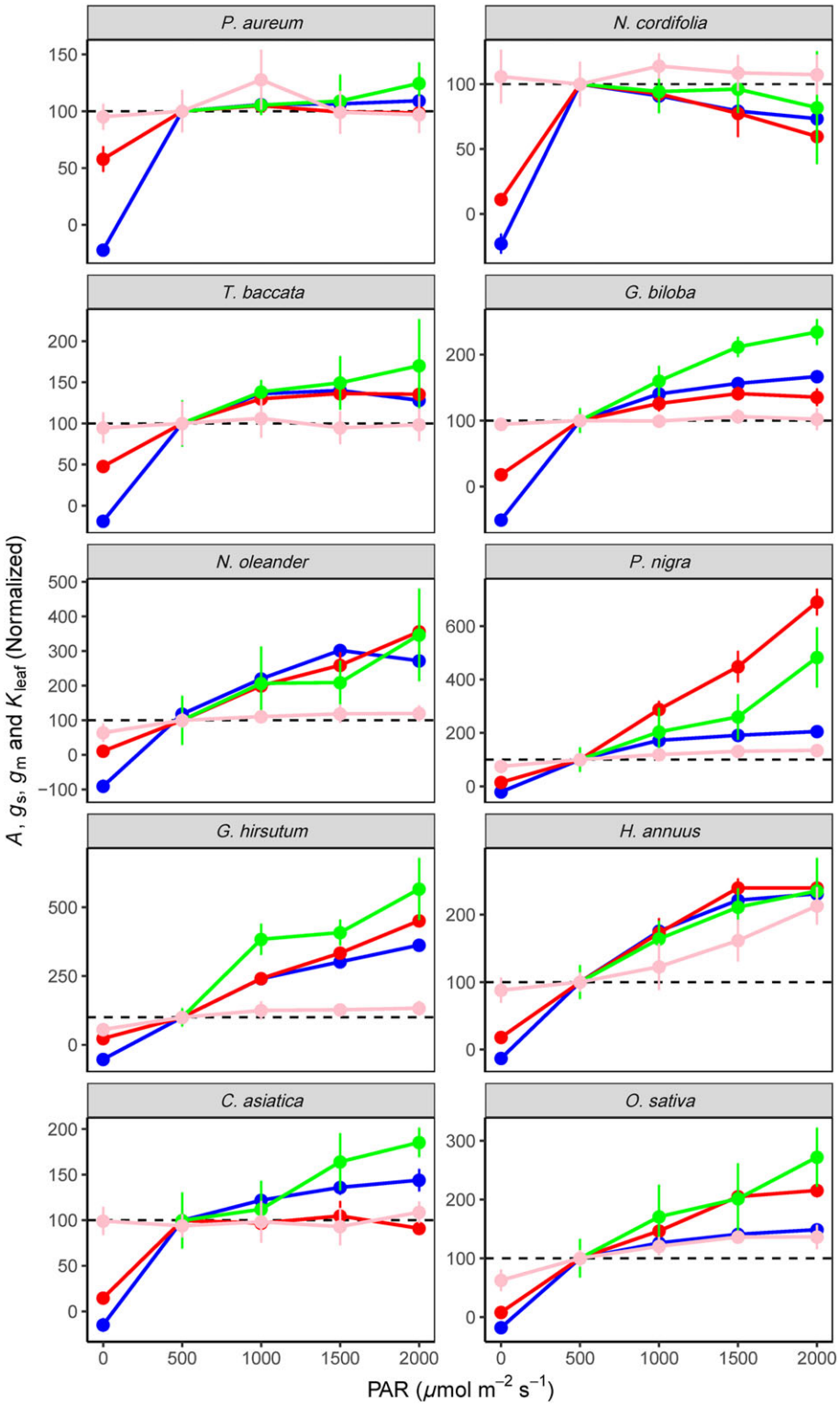

FIGURE 6 Patterns of light responses of photosynthetic rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), and leaf hydraulic conductance (K_{leaf}). The parameters were normalized to average values at PAR = 500 μmol·m^{-2}·s^{-1} ($X_{Normalized}$ = 100%*(X_i/X_{500}), where $X_{Normalized}$ is the normalized value, X_i is the value at PAR = i μmol·m^{-2}·s^{-1}, and X_{500} is the value at PAR = 500 μmol·m^{-2}·s^{-1}). Blue, red, green, and pink points are normalized A, g_s, g_m, and K_{leaf}, respectively. Data are the means ± SD (n = 3–6) [Colour figure can be viewed at wileyonlinelibrary.com]

movement in the transition from light to darkness, which then cause faster stomatal closing. Because both passive and active ways to control stomatal movements in gymnosperms are important, the opening speed in this group is more variable. Interestingly, we also found that the stomatal closing of *C. asiatica*, a wet habitat angiosperm, was slower than other angiosperms and even gymnosperms. This was associated with an absence of stomatal control under light changes (though stomata still opened and closed in response to light and darkness), suggesting that stomatal closing could be different in species from wet habitats. It is important to note that more species need to be measured

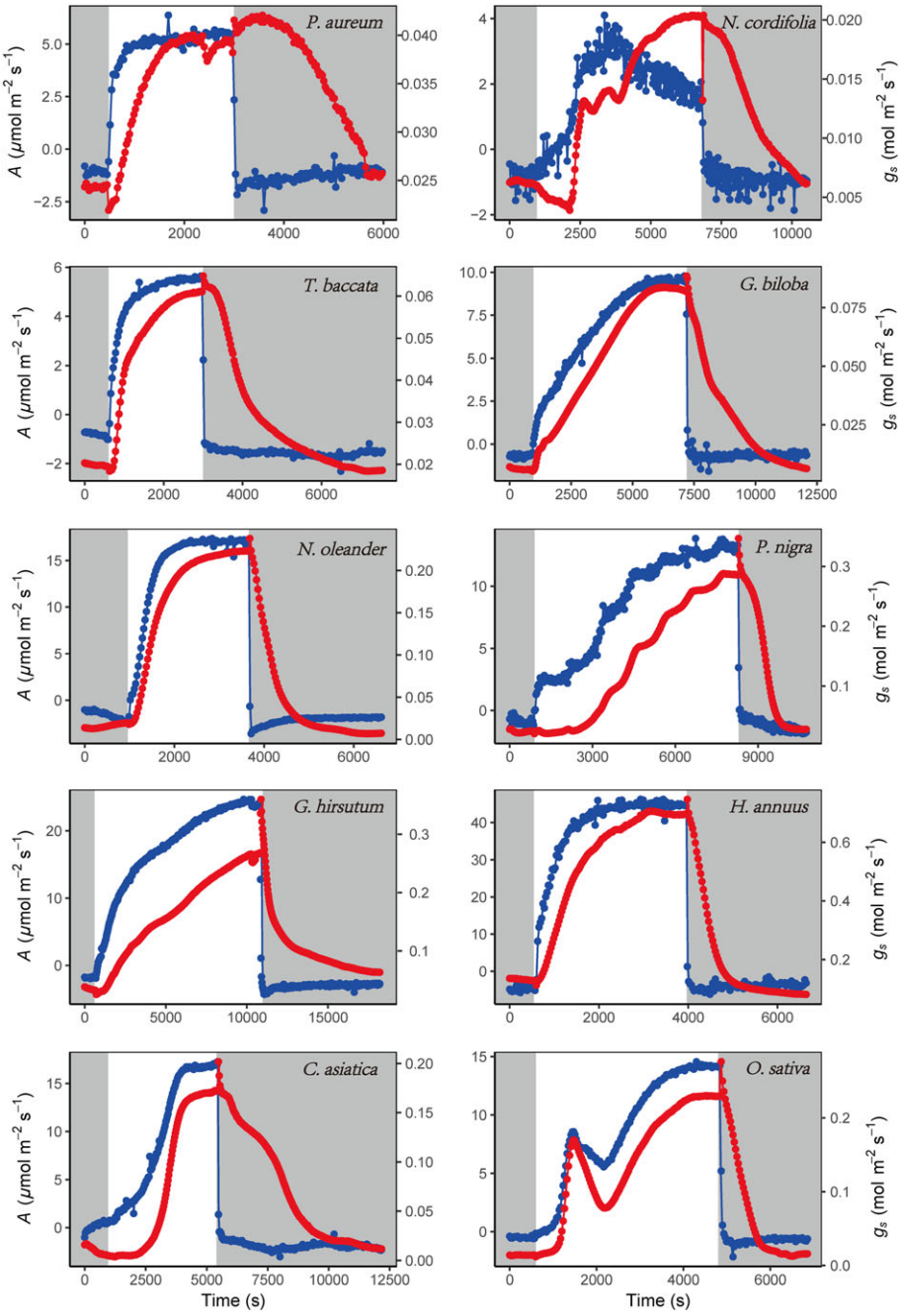

FIGURE 7 Typical time responses of photosynthetic rate (A) and stomatal conductance (g_s) to light changes in each species. Grey shading represents darkness, and white represents 1,500 μmol·m^{-2}·s^{-1}. Blue and red points are A and g_s, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

to confirm those trends and definitively conclude that these results indicate evolutionary patterns rather than species differences.

Previous works have suggested that small stomata have a faster speed of opening and closing than large ones, and the evolutionary trend from a few large stomata to high density stomata is assumed to represent greater efficiency in stomatal movement under natural condition (Drake, Froend, & Franks, 2013; Lawson & Blatt, 2014; Raven, 2014). Although a few studies have estimated the correlation between stomatal size and stomatal response speed, there is no consistent conclusion. A negative correlation between stomatal size and maximum rate of stomatal opening was observed in the genus *Banksia* (Drake et al., 2013), but other studies showed no correlation (Elliott-Kingston et al., 2016; Haworth, Killi, Materassi, Raschi, & Centritto, 2016) or even a positive correlation (Monda et al., 2016). Indeed, there is a clear evolutionary trend in stomatal size and density. To our knowledge, no previous study has estimated stomatal response rates to both light and darkness with multiple phylogenetically distant species. We found that the half-time of stomatal closing was correlated with stomatal size but not with stomatal density; moreover, the half-time stomatal opening was independent of both stomatal size and density. Our results suggest that stomatal size is not the key factor for the speed of g_s response to light, though it could be related to stomatal closing speed.

In conclusion, these results provide new clues for understanding of the coordination of leaf hydraulic conductance and gas exchange.

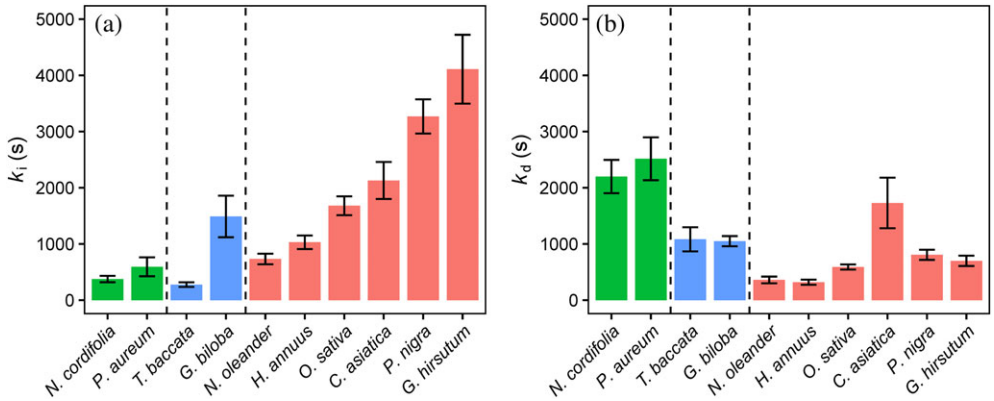

FIGURE 8 Mean ± *SD* (n = 3) values of (a) time to 50% increase of g_s under 1,500 μmol·m^{-2}·s^{-1} PAR (k_i) and (b) time to 50% decline of g_s after imposing darkness (k_d). Green, blue, and red colors represent ferns, gymnosperms, and angiosperms, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Presence (yes) or absence (no) of the response of photosynthetic parameters to variation in light intensity (from 2,000 to 500 μmol·m^{-2}·s^{-1}), or from light to darkness

	Light			Darkness	
Group	g_s	g_m	K_{leaf}	g_s	K_{leaf}
Ferns	No	No	No	Yes	No
Gymnosperms	Yes	Yes	No	Yes	No
Angiosperms	Yes	Yes	Yes	Yes	Yes
Centella asiatica	No	Yes (but low)	No	Yes	No

Note. Either for light variation or transition light to darkness, a steady-state of minimum 0.5 hr was imposed. The presence/absence of response is detailed for each phylogenetic group, with "*Centella asiatica*," a wet habitat angiosperm (Apiaceae), treated as an outlier.

In particular, (a) a phylogenetic trend (although more species should be studied to confirm it) emerges from ferns to angiosperms, consisting of increasing steady-state values for K_{leaf}, A, g_s, and g_m, associated with increasing VLA; (b) a similar phylogenetic trend is observed concerning the response of these parameters to varying light, with a gradual increase in the number of traits able to respond to light from none in ferns to all in angiosperms; (c) these phylogenetic trends may have exceptions, as illustrated here by the angiosperm species *C. asiatica*, which behaves in all studied aspects similar to a fern; and finally, (d) differences among phylogenetic groups are also evidenced concerning in their stomatal kinetics, with stomatal opening faster in ferns and gymnosperms than angiosperms, but stomatal closing faster in angiosperms. This likely reflects different mechanisms or combinations of mechanisms for the regulation of stomatal movements in each phylogenetic group.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Light response curves of the ten species. Data were shown as mean ± SD (n = 3–6).

Figure S2. Correlations of light saturated photosynthetic rate (A_{sat}) with (A) stomatal conductance (g_{s_sat}) and (B) mesophyll conductance (g_{m_sat}). Values are mean ± SD (n = 3–6).

Figure S3. Mean values of theoretical maximum stomatal conductance (g_{s_max}) of each species. The values are mean ± SD.

Figure S4. Response of stomatal conductance (g_s) to light intensities in the ten species. Data were shown as mean ± SD (n = 3–6).

Figure S5. Response of mesophyll conductance (g_m) to light intensities in the ten species. Data were shown as mean ± SD (n = 3–6).

Figure S6. Response of leaf hydraulic conductance (K_{leaf}) to light intensities in the ten species. Data were shown as mean ± SD (n = 3–6).

Figure S7. Response of leaf water potential to light intensities in the ten species. Data are shown as means ± SD (n = 3–6).

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