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REVIEW PAPER

Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination

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Abstract

Two highly contrasting variables summarizing the efficiency of transport of materials within the leaf are recognized as playing central roles in determining gas exchange and plant performance. This paper summarizes current approaches for the measurement of mesophyll conductance to CO_2 (g_m) and leaf hydraulic conductance (K_{leaf}) and addresses the physiological integration of these parameters. First, the most common methods to determine g_m and K_{leaf} are summarized. Next, novel data compilation is analysed, which indicates that, across diverse species, g_m is strongly linked with gas exchange parameters such as net CO_2 assimilation (A_{area}) and stomatal conductance (g_s), and with K_{leaf}, independently of leaf vein length per leaf area. Based on their parallel responses to a number of environmental variables, this review proposes that g_m is linked to the outside-xylem but not to the xylem component of K_{leaf}. Further, a mechanistic hypothesis is proposed to explain the interactions among all these and other physiological parameters. Finally, the possibility of estimating g_m based on this hypothesis was tested using a regression analysis and a neurofuzzy logic approach. These approaches enabled the estimation of g_m of given species from K_{leaf} and leaf mass per area, providing a higher predictive power than from either parameter alone. The possibility of estimating g_m from measured K_{leaf} or vice-versa would result in a rapid increase in available data. Studies in which g_m, K_{leaf}, and leaf mass per area are simultaneously determined are needed in order to confirm and strengthen predictive and explanatory models for these parameters and importantly improve resolution of the integrated hydraulic-stomatal-photosynthetic system.

Key words: Coordination traits, leaf hydraulic conductance, leaf mass area, mesophyll conductance, photosynthesis, vein leaf area.

Introduction

The mesophyll conductance to CO_2 assimilation (g_m; mol CO_2 m^{-2} s^{-1}) and the leaf hydraulic conductance (K_{leaf}; units mmol m^{-2} s^{-1} MPa^{-1}) are two variables that importantly influence transport of materials within the leaf. These two variables have been increasingly recognized as playing central roles in determining gas exchange rates and plant performance. However, while both traits have independently been the focus of a large number of studies in the past two decades, very few have focused on their coordination. In this work, the aims were 3-fold: (i) to summarize current approaches and point to key resources for the measurement of g_m and K_{leaf}; (ii) to present theory and a novel data compilation showing their physiological integration; and (iii) to indicate the importance of frontier research based on quantifying both variables.

Despite the fact that both g_m and K_{leaf} are named as 'conductances', g_m and K_{leaf} could not be more different in what they represent. These traits quantify the effectiveness of the transport of different substances, in partially distinct leaf tissues, by different physical processes, and are estimated with completely different measurement techniques. Thus,

g_m represents the conductance of the diffusional pathway of gaseous CO_2 from the substomatal cavity through the mesophyll tissue into the cells and then into the chloroplasts where biochemical assimilation occurs (Flexas *et al.*, 2012a,b). K_{leaf} represents the conductance of the bulk flow pathway of liquid water from the petiole through the xylem throughout the leaf and across the bundle sheath surrounding the veins, through or around cells to the sites of evaporation (Tyree and Zimmermann, 2002; Sack and Holbrook, 2006). g_m is measured using gas exchange systems in combination with other methods, whereas K_{leaf} is typically measured using hydraulic apparatus. While g_m is known to be composed of the conductances of a number of components of the diffusional pathway, including those of the intercellular air spaces (g_{ias}), cell walls (g_{cw}), and chloroplast outer membrane (g_{chl}), among others (Flexas *et al.*, 2012b), in practice it has not yet been directly decomposed into its fractions due to current technical limitations. By contrast, K_{leaf} has been considered in terms of its subcomponents, the conductance of the outside-xylem pathways (K_{ox}) (including apoplastic, symplastic, and cell-to-cell pathways for water movement), and the xylem pathways (K_x; i.e. the pathways inside the veins, both of which are strongly influenced by the vein length per leaf area, *VLA*, also known as 'vein density'; reviewed in Sack and Scoffoni, 2013a). These parameters—g_m, K_{leaf}, K_{ox}, K_x, and *VLA*—are those discussed in detail most in this paper, with other physiological and structural traits being briefly defined at their first appearance.

Despite their representing the transport of contrasting substances according to different physics, both g_m and K_{leaf} contribute strongly to the determination of maximum rates of photosynthesis and its dynamics under shifting environmental conditions, and they partly share a mechanistic basis. In reviewing the measurement techniques and analysing what can be derived from their integration, this work promotes a new approach towards clearer resolution of the complete hydraulic-stomatal-photosynthetic system that fuels plant growth. This review also highlights that the concept of 'optimization' of water use and carbon gain now needs to consider xylem and outside-xylem flow pathways, and within-leaf gas phase delivery to chloroplasts.

Current approaches to the measurement of mesophyll conductance (g_m)

There are currently three major approaches commonly used for the estimation of g_m, although other methods have been described (see details in Warren, 2006; Pons *et al.*, 2009; Flexas *et al.*, 2012a). These three current approaches are (i) the on-line carbon isotope discrimination method (i.e. the 'Evans' method, after Evans *et al.*, 1986); (i) the combined chrorophyll fluorescence and gas exchange method (i.e. the 'Harley' method, after Harley *et al.*, 1992); and (iii) the curve-fitting method (i.e. the 'Ethier' method, after Ethier and Livingston, 2004). All three methods have advantages and drawbacks.

The Evans method is based on the idea that the discrimination of Rubisco against ^{13}C depends on the CO_2 concentration at the carboxylation sites inside the chloroplasts (C_c). When using common infra-red gas analysers (IRGAs), the CO_2 concentration in intercellular air spaces (C_i) is estimated; thus, one can estimate a 'theoretical' discrimination ($\Delta^{13}C_i$) assuming that $C_i=C_c$. The difference between $\Delta^{13}C_i$ and the actual measured discrimination ($\Delta^{13}C_r$) can be then used to estimate C_c, which enables estimation of g_m, as $g_m=A_N/(C_i–C_c)$, where A_N is the net CO_2 assimilation determined with the IRGA. This method requires the use of a mass spectrometer or a tuneable laser diode for the measurement of $\Delta^{13}C_r$, coupled to an IRGA for the measurement of A_N and C_i, and *a priori* knowledge of carbon isotope discrimination throughout the CO_2 assimilation process: that is, (i) during CO_2 diffusion in air from outside the leaf through stomata into the intercellular air spaces; (ii) during Rubisco and PEPcase carboxylation (and the relative rate of the two reactions); and (iii) during respiration and photorespiration. All these values are considered as constants, which introduces uncertainty in the estimation, because potential differences across species and/or experimental conditions have not yet been fully quantified (Flexas *et al.*, 2012a). Another drawback of this method is that it cannot be used directly under field conditions, although the method may be applied by collecting air from gas exchange chambers in the field into flasks and later performing an 'off-line' discrimination analysis in the laboratory, or by using the discrimination of recently synthesized sugars after their extraction in the laboratory (see Flexas *et al.*, 2012a for details). Further, the precision of tuneable laser diodes and mass spectrometers (except dual-inlet configurations) is often too low for the reliable determination of the carbon isotope discrimination of a small area of leaf, and thus larger-than-typical gas exchange cuvettes are generally required (Pons *et al.*, 2009; Flexas *et al.*, 2012a). The main advantage of this method is that it is the only one based on combining information from two completely independent measurements (gas exchange and carbon isotope discrimination) determined from the exact same photosynthetic tissue.

The Harley method is based on the assumption that all the energy and reducing power generated by the electron transport reactions in the chloroplast thylakoid membranes is used to fuel either photosynthetic carboxylation or oxygenation (i.e. photorespiration), while their use in other processes (e.g. photosynthetic nitrate reduction, Mehler reaction) is negligible. The g_m can thus be estimated from measurements of the rate of electron transport (ETR) using a chlorophyll fluorimeter, and measurements of the A_N and the rate of day respiration using an IRGA. The ETR is used to estimate the gross photosynthetic rate, and hence the net CO_2 assimilation can be used to estimate the fractions of thylakoid energy used in carboxylation and oxygenation, and then the C_c can be estimated using *a priori* knowledge of the Rubisco specificity factor or the CO_2 photocompensation point (Γ^*) and other factors that may be obtained using independent methods (further details can be found in Warren, 2006; Pons *et al.*, 2009; and Flexas *et al.*, 2012a). A main drawback of this method is that ETR is estimated from the fluorescence of the top cell layers of the leaf, while photosynthetic gas exchange is measured from the bulk leaf enclosed in the IRGA cuvette. Thus, basing a g_m estimate on these two mismatched measurements can introduce uncertainty, especially in thick leaves. Another drawback of this approach is the requirement of many additional measurements for the estimation of g_m, such as leaf absorpance (required for accurate estimation of ETR), day respiration, and Γ^*. The main advantage of this method is that it is easier to use under field conditions, while still combining two independent measurements for the estimation of g_m (Pons *et al.*, 2009; Flexas *et al.*, 2012a).

Finally, the Ethier method is based on the idea that a finite g_m increases the curvature of the graphical plot of the measured response of A_N to C_i (i.e. the A–C_i curve). Using a specifically designed curve-fitting based on the Farquhar *et al.* (1980) model of photosynthesis, one can estimate an average g_m in addition to the standard parameters yielded from A–C_i curve analysis (i.e. the maximum velocities of carboxylation and electron transport; respectively V_{cmax} and J_{max}). The obvious advantage of this method is that it only requires the use of an IRGA. However, a drawback of this method, as for the Evans method, is the need for *a priori* knowledge of Γ^* and other Rubisco kinetics and their temperature responses, all of which are typically treated as constants based on the values reported for tobacco by Bernacchi *et al.* (2002), thus not accounting for potential differences across species and/or experimental conditions. The other major drawback of this method is its statistical extraction of values for at least three unknowns (g_m, V_{cmax}, and J_{max}) from a single curve-fitting exercise, which it is often statistically weak, unless one has exceptionally clean and smooth A–C_i curves with a large number of points or C_i intervals.

Because all three methods present a number of drawbacks and require many assumptions that are often not fully tested, several have recommended the use of at least two methods simultaneously to report differences in g_m among species or treatments (Pons *et al.*, 2009; Flexas *et al.*, 2012a).

Current approaches to the measurement of leaf hydraulic conductance (K_{leaf})

The leaf hydraulic conductance (K_{leaf}=1/leaf hydraulic resistance) is determined as the ratio of the water flow rate through the leaf to the water potential gradient driving force for water movement across the leaf. The K_{leaf} summarizes the behaviour of a complex system: water moves through the petiole and through several orders of veins, exits into the bundle sheath and passes through (via symplastic or cell-to-cell pathways) or around (via apoplastic pathway) mesophyll cells before evaporating into the airspace and being transpired from the stomata (Sack and Tyree, 2005; Sack and Holbrook, 2006). The K_{leaf} has been quantified for over 20 years, with a dramatic increase since the late 1990s, and a number of different approaches have been used based on different techniques. Currently, most researchers measure K_{leaf} for excised leaves, typically using one of four methods: (i) the evaporative flux method (EFM); (ii) the rehydration kinetics methods (RKM); (iii) the high pressure flowmeter (HPFM); and (iv) the vacuum pump method (VPM). A minority of researchers use (v) *in vivo* methods for estimating K_{leaf}. All these methods have advantages and drawbacks in terms of logistics, field portability, biological realism, and precision.

The EFM is based on driving transpiration (E) for a leaf with petiole attached to a potometer, and when flow (and leaf water potential, Ψ_{leaf}) reaches a steady state, subsequently determining the water potential driving force across the leaf ($\Delta\Psi_{leaf}$), with $K_{leaf}=E/\Delta\Psi_{leaf}$. Resources for describing EFM measurement include Sack *et al.* (2002), Brodribb *et al.* (2007), Scoffoni *et al.* (2008, 2012), Guyot *et al.* (2012), and a recent movie (Sack and Scoffoni, 2013b). A drawback of the EFM is its relying on estimating the water potential driving force for water movement from equilibrated, non-transpiring leaf tissue, using the pressure chamber (or another technique, for example psychrometry) which introduces some uncertainty, as the true driving force during transpiration may be stronger, by an amount that depends on where in the leaf the water is evaporating and the precise transpiration pathways, which are largely unknown (reviewed by Sack *et al.*, 2002; Sack and Tyree, 2005; Sack and Holbrook, 2006; Guyot *et al.*, 2012; Scoffoni *et al.*, 2012). The main advantages of the EFM are its simplicity, practicality, and realism, as the transpiration of the excised leaf is just as *in vivo*, driven by the vapour pressure deficit (VPD) of the ambient air. Additional advantages are the ability to manipulate light, and potentially temperature and humidity around the leaf, and also to estimate stomatal conductance (g_s) during the measurement (Guyot *et al.*, 2012; Sack and Scoffoni, 2013b). A measurement can be made in 30–60 min.

The RKM is based on estimating the K_{leaf} during the uptake of water into a partially dehydrated leaf. In an early version of this method, K_{leaf} was estimated from (i) the initial Ψ_{leaf} determined from equilibrated adjacent leaves, (ii) the final Ψ_{leaf} after rehydration, (iii) the rehydration time, and (iv) the water uptake during rehydration, which was in turn estimated from the water potential measurements, and the leaf water storage capacitance estimated from pressure volume curves; resources for that measurement include Brodribb and Holbrook (2003) and Brodribb *et al.* (2005). A more recent, refined version of the RKM, the 'Dynamic-RKM', involves directly measuring the water uptake during rehydration, rather than estimating this based on the capacitance. Resources for this measurement include Brodribb and Cochard (2009), Blackman and Brodribb (2011), and Brodribb *et al.* (2011). A drawback of either version of the RFM is its relying on estimating the water potential driving force for rehydration from equilibrated leaf tissue which introduces some uncertainty, as the true driving force during rehydration may be stronger. Further drawbacks include uncertainty in some of the inputs into the calculation, such as noisiness in the data arising from determining initial and final Ψ_{leaf} from different, albeit approximately equilibrated leaves and the precise rehydration time (since the leaf mesophyll may continue to rehydrate even after the petiole is removed from water). Additional drawbacks of this approach include the uncertainty of the flow pathways during rehydration and the degree that these pathways mimic those of transpiration. The advantage of this measurement is its rapidity, as measurements can be made within minutes.

The HPFM typically involves forcing water through a system of tubing including a high-resistance segment of tubing placed between two pressure transducers, through the petiole, into a leaf until it leaks from the stomata and a steady state flow is established. The K_{leaf} is measured using the known pressure applied and the drop in pressure in the high-resistance tubing relative to that across the leaf (resources include Yang and Tyree, 1994; Sack *et al.*, 2002; Tyree *et al.*, 2005). Drawbacks include the inability to measure partially dehydrated leaves, which is often desirable to determine the dynamics of K_{leaf} with dehydration. Further, this measurement approach involves artificial conditions arising in the leaf, including the flooding of mesophyll and forcing of water through the airspaces out of the stomata. The question has been raised of whether stomatal closure might influence the measurement (Sack *et al.*, 2002; Rockwell *et al.*, 2011) but detailed tests indicated that this was a negligible factor (Sack *et al.*, 2002; Tyree *et al.*, 2005; Cochard *et al.*, 2007; Nardini *et al.*, 2010). Rockwell *et al.* (2011) also hypothesized that the flooding of the mesophyll would possibly reduce the K_{leaf}, but data have not been conclusive. A major advantage of this method is the ease of controlling light and/or temperature, as the leaf can be maintained submerged in a temperature-controlled water bath. A measurement can be made in 30–60 min. Several modified versions of this approach have been developed using the pressure chamber to drive flow, either by forcing water out of the leaf lamina cells through the petiole protruding from the chamber (Franks, 2006), or by submerging a shoot underwater in the pressure chamber and forcing water through the stomata into the mesophyll, and thereby into the xylem and eventually out of the cut shoot protruding from the chamber (Postaire *et al.*, 2010; Prado *et al.*, 2013).

The VPM is based on drawing water out of the leaf using subatmospheric pressures and determining K_{leaf} as the slope of flow rate against pressure (Martre *et al.*, 2001; Nardini *et al.*, 2001; Sack *et al.*, 2002). Drawbacks of this method are lack of realism in subatmospheric pressure as a driving force for water flow within the leaf airspaces, a lack of field portability and lengthy measurement times (up to 2 h per leaf). Further, some leaves show non-linearities of flow against pressure, implying changes in flow pathways within the leaf, or damaged cells (e.g. Martre *et al.*, 2001).

Notably, while the HPFM and VPM are least realistic and have drawbacks, a major advantage of these methods is the ability to calculate K_{leaf} directly, rather than as a ratio of two separate measurements as occurs for EFM and dynamic RKM (i.e. from E and $\Delta\Psi$). Consequently, the data tend to be less noisy and fewer replicates are often necessary (e.g. 5–6 for HPFM or VPM; whereas often >10 are required for EFM and RKM). Thus, if few leaves are available for measurement, e.g. due to species rarity or complex experimental treatments, then these methods can be especially attractive.

In vivo measurements of K_{leaf} have been made (i.e. leaf transpiration (E) determined while still attached to the plant). The E can be estimated from sapflow methods or gas exchange measurements, and the water potential driving force estimated as the difference between bagged and unbagged leaf water potential (reviewed in Sack and Tyree, 2005). *In vivo* measurements can be subject to instability, without clear steady state transpiration, and thus are frequently noisy. Further, these methods do not allow the control of environmental conditions as for methods applied to excised leaves. A major challenge for ongoing methods development is an *in vivo* method that is accurate and reliable, which would enable the characterization of K_{leaf} dynamics in real time and *in vivo*, rather than on excised leaves (cf. Scoffoni *et al.*, 2012).

The integration of mesophyll conductance and leaf hydraulic conductance

Meta-analysis of the coordination of g_m *and* K_{leaf}

The g_{m} and K_{leaf} might be expected to be coordinated, due to their both being critically involved in leaf function and determinants of photosynthetic rate, and further they may depend in part on some shared structural and/or physiological traits, leading to a partially integrated mechanistic basis. Thus, Griffiths and Helliker (2013) suggested that water transport efficiency, as determined by hydraulic supply and regulation by stomata, might influence g_{m}, but the question has not been investigated directly with data. Using a newly compiled dataset for diverse species (Supplementary Table S1, available at *JXB* online) for g_{m}, K_{leaf}, stomatal conductance (g_{s}), and light-saturated photosynthetic rate, the current work found novel trends for a coordination among these traits on both a leaf area- and a leaf mass-normalized basis (Fig. 2A–E). In this dataset was found the coordination of g_{m}, g_{s}, and photosynthetic rate per leaf area (A_{area}) previously reported in a number of recent papers (Fig. 1A and B; see Flexas *et al.*, 2002, 2004, 2012b, 2013). There was also a strong correlation of A_{area} with K_{leaf} (Fig. 2A and C), as has been previously reported across species (Brodribb and Holbrook, 2006; Franks, 2006; Walls, 2011). Only a non-significant empirical trend was found between K_{leaf} and g_{s} in these data, although such a relationship has been reported previously (Franks, 2006). The current work also discovered a correlation of g_{m} with K_{leaf} with across diverse species. A previous heuristic indication that a relationship of g_{m} with K_{leaf} might exist was provided by Flexas *et al.* (2012b), who had found a correlation across diverse species of g_{m} with hydraulic conductances of various organs, for related species within the same genus or family, when excluding a number of outliers. This new analysis shows that this trend holds strongly across diverse dicot species. These trends were all strengthened by placing traits on a leaf-mass basis (Fig. 2D–F; see following section for additional discussion of area- and mass-based normalization).

Implications of the coordination of K_{leaf} with g_{m} exist at multiple scales. The correlation of K_{leaf} with g_{m} but not significantly with g_{s} in this work's dataset implies a shared mechanistic basis for trait coordination (as will be discussed). The typical explanation for the correlation of K_{leaf} with g_{s} and A_{area} across species is based on optimal hydraulic design, such that plants should have a K_{leaf} that matches their g_{s} to match hydraulic supply with demand, when operating within a narrow range of soil water potential, leaf water potential,

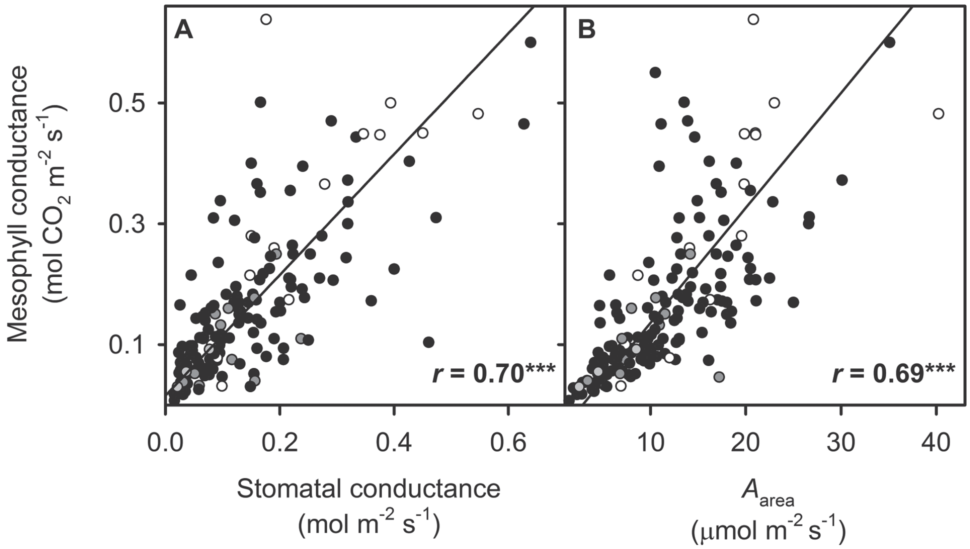

Fig. 1. Coordination of mesophyll conductance (g_m) with (A) stomatal conductance (g_s) and (B) maximum assimilation rate per area (A_{area}) across diverse species in a compiled database. Gymnosperms (dark grey circles; n=12), ferns (light grey circles; n=3), monocots (open circles; n=14), and dicots (filled circles; n=144) are represented. Standard major axes were fitted through the dicotyledons dataset only. (A) g_m=0.999g_s+0.016; (B) g_m=0.019A_{area}–0.054. ***P<0.001.

and VPD (to be explained further). However, across species adapted to varying environments, such correlation of K_{leaf} with g_s would be expected to be weak. The correlation of K_{leaf} with g_m across species implies an additional co-selection of high g_m to enhance greater CO_2 assimilation to optimize performance for species with high K_{leaf} and g_s to enable higher rates of gas exchange. This finding suggests that, while historically it has been thought that water use and assimilation are coordinated within the plant due simply to their co-regulation by stomata, that the concept of 'optimization' of water use and carbon gain now needs to consider xylem and outside-xylem flow pathways, and within-leaf gas phase delivery to chloroplasts. Indeed, this correlation of K_{leaf} and g_m indicates the possibility of partial overlap in their mechanistic basis (i.e. due to their both depending on mesophyll structure and physiology, as will be discussed).

The independence of the coordination of K_{leaf} with g_m from the xylem was highlighted by the non-correlation of K_{leaf} with *VLA* ('vein density') in these compiled data (Fig. 3). The *VLA* is an important underlying factor in the hydraulic conductance of both the xylem (K_x) and outside-xylem pathways (K_{ox}), since a higher *VLA* will increase xylem pathways in parallel and reduce the flow pathways outside the xylem via apoplastic, symplastic, and/or cell-to-cell pathways (Sack and Scoffoni, 2013a). In other datasets, *VLA* has been hypothesized to be a strong, or even the major, driver of variation across species in K_{leaf} and thus, the main determinant of the correlation of K_{leaf} with gas exchange (Sack and Frole, 2006; Brodribb *et al.*, 2007; Boyce *et al.*, 2009). However, multiple traits in addition to *VLA* can influence both K_x and K_{ox}. For example K_x also depends importantly on the number and sizes of conduits within the veins, and in particular in the major veins (McKown *et al.*, 2010; Sack and Scoffoni, 2013a). Further, the K_{ox} depends additionally on mesophyll structure and physiology (Sack and Scoffoni, 2013a; to be discussed further). Thus, in these data, it appeared that K_{ox} was a major influence on K_{leaf} and independent of *VLA*, and that K_{leaf} correlated with g_m due to their influence by a common factor, and that this contributed in part to the correlation of K_{leaf} with A_{area}, a point to be further discussed.

Coordination on a leaf-area versus leaf-mass basis

Traditionally, gas exchange variables, g_m included, have been normalized by dividing by leaf area but also often expressed on a per leaf-mass basis by dividing the area-normalized rates by leaf mass per area (*LMA*). By contrast, hydraulic supply to leaves has been traditionally only expressed on a leaf-area basis; however, recent studies have begun also to normalize K_{leaf} on a leaf-mass basis ($K_{leaf,mass}$; Niinemets and Sack, 2006; Nardini *et al.*, 2012; Simonin *et al.*, 2012). Recent work has demonstrated that some trait correlations, and in particular those among 'leaf economics' variables expressed on a mass basis, such as photosynthetic rate per leaf mass (A_{mass}), nitrogen concentration per leaf mass (N_{mass}), and *LMA*, arise in part automatically, or by statistical necessity, given their basis of expression per unit leaf area or per unit leaf dry mass (Lloyd *et al.*, 2013; Osnas *et al.*, 2013). Thus, correlations among mass-based traits and *LMA* may result when mass-based traits are determined by dividing area-based traits by *LMA* (e.g. $K_{leaf,mass}$=K_{leaf}/*LMA*). Similarly, correlations among area-based traits and *LMA* may result when area-based traits are determined by multiplying mass-based traits by *LMA* (e.g. N_{area}=N_{mass}×*LMA*) (Lloyd *et al.*, 2013; Osnas *et al.*, 2013; Sack *et al.*, 2013). This review follows Sack *et al.* (2013) in referring to such correlations as 'innate correlations'. These linkages may arise due to the calculation of variables, but still represent physical phenomena important to plant function. Leaves built

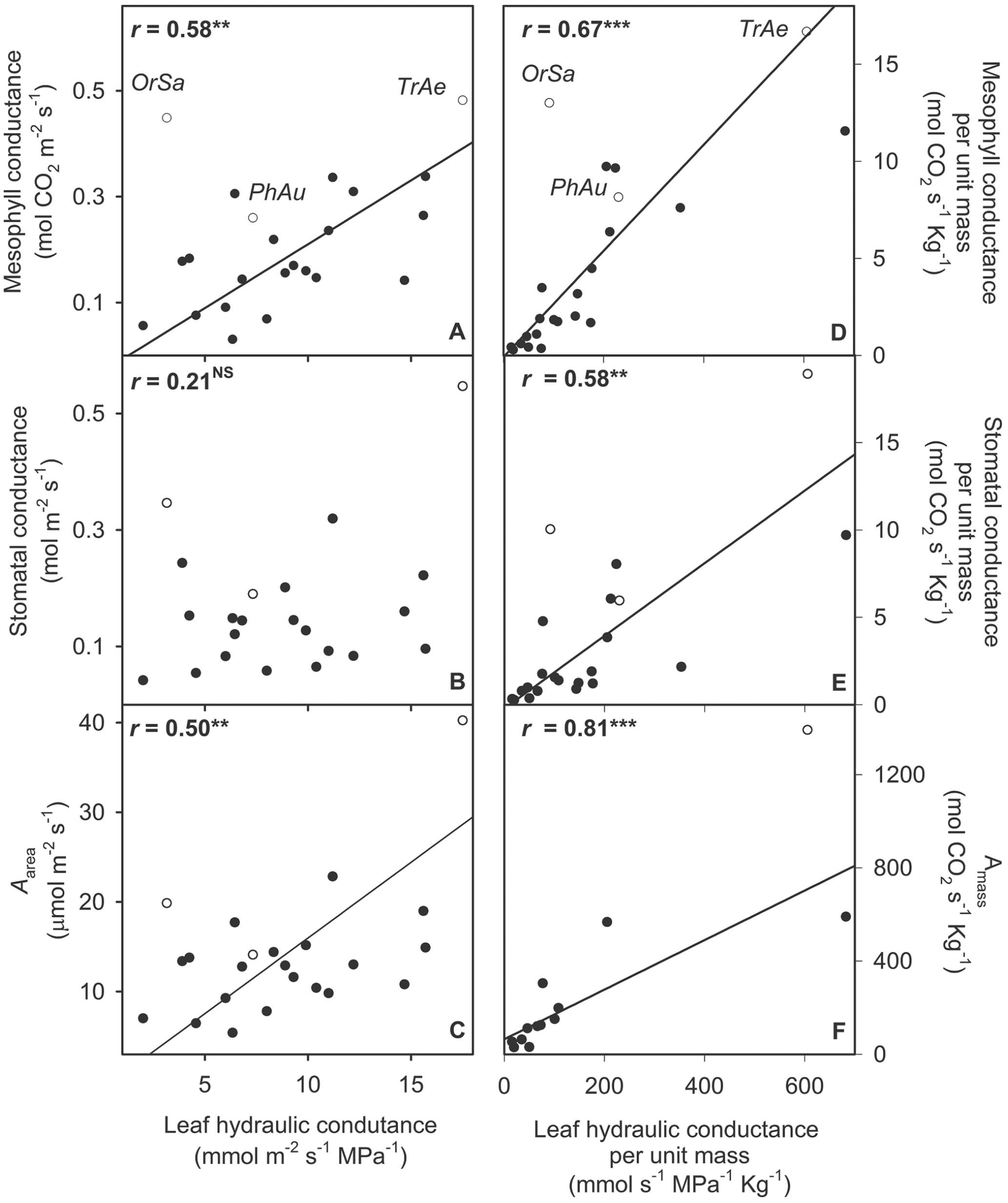

Fig. 2. Coordination of mesophyll conductance (g_m), stomatal conductance (g_s), and maximum assimilation rate (*A*) with leaf hydraulic conductance (K_{leaf}) on an area (A–C) and mass basis (D–F) across angiosperm species in a compiled database. Monocotyledons [open circles; *n*=3: *Oryza sativa* (*OrSa*), *Phragmites australis* (*PhAu*), and *Triticum aestivum* (*TrAe*)] and dicotyledons (filled circles; *n*=20) are represented. Standard major axes are fitted through the data for dicotyledons. (A) g_m=0.025K_{leaf}–0.031; (B) no significant correlation was found; (C) g_m=1.69K_{leaf}–0.92; (D) $g_{m,mass}$=0.027$K_{leaf,mass}$–0.072; (E) $g_{m,mass}$=0.021$K_{leaf,mass}$–0.022; (F) A_{mass}=1.06$K_{leaf,mass}$–64.7. NS, P>0.05; **P<0.01; ***P<0.001.

of cells with thicker cell walls will tend to have higher *LMA* and to have 'diluted' physiological rates and conductances that are expressed on a mass basis, and thus lower A_{mass} and $K_{leaf,mass}$. The resulting negative correlations of $K_{leaf,mass}$ and A_{mass} with *LMA* relevant to leaf economic performance: leaves with high *LMA* will yield less return in hydraulic conductance and photosynthetic rate over short timescales but have benefits over long leaf lifetimes (Westoby *et al.*, 2013). Thus, even if they arise in part innately, relationships among mass-based traits are considered by most to represent mechanistic trait linkages with ecological significance, especially given that mass-based traits scale up most directly to influencing whole-plant relative growth rate (to be discussed). Thus, the correlation of K_{leaf} with g_m on a mass basis (Fig. 2D–F) indicates a coordination that would scale up to influencing whole plant carbon budgets.

Common dynamic responses of g_m *and* K_{leaf}

The previous section showed the coordination of steady-state, maximum values of g_m and K_{leaf} (i.e. under non-stress conditions). However, both parameters are dynamic at a

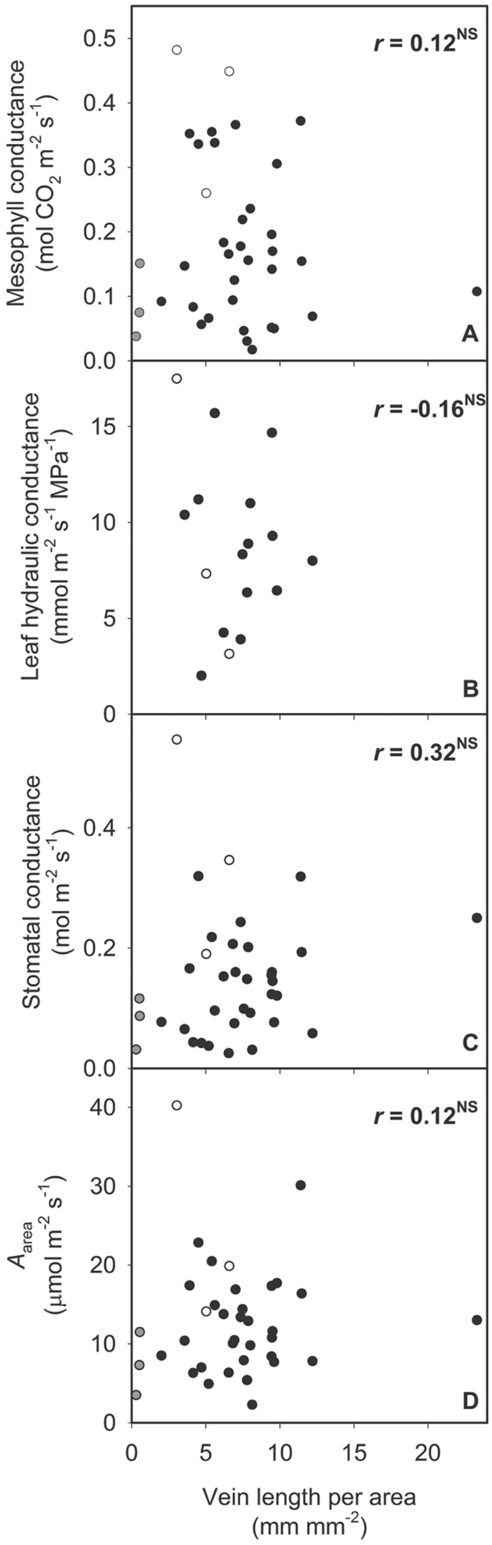

Fig. 3. Independence of (A) mesophyll conductance, (B) leaf hydraulic conductance, (C) stomatal conductance, and (D) maximum assimilation rate per area (A_{area}) from leaf vein length per area across diverse species in a compiled database. Gymnosperms (grey circles; n=3 in A, C, and D), monocots (open circles; n=3 in A, C, and D), and dicots (filled circles; n=31, 14, 30, and 31 in A, B, C, and D, respectively) are represented. Correlation coefficient and significance were obtained from standard major axes fitted through the dicot dataset only. NS, P>0.05.

range of time scales from minutes to hours to days to seasons to years (Sack and Holbrook, 2006; Flexas *et al.*, 2007, 2008). The short-term dynamics (seconds to hours) of both variables have attracted increasing attention because of their likely ability to influence instantaneous and daily means for water use and photosynthetic carbon gain.

Indeed, g_m and K_{leaf} show responses which are similar in direction and extent to a number of environmental variables (Table 1). In response to increased leaf temperature, g_m increases to an optimum, depending on the species and acclimation conditions, and then decreases thereafter (Bernacchi *et al.*, 2002; Warren and Dreyer, 2006; Yamori *et al.*, 2006; Warren, 2008a), although the latter decrease has been debated (Evans and von Caemmerer, 2013). The g_m also shows a longer-term acclimation, i.e. for plants growing and producing leaves under different temperature regimes (Yamori *et al.*, 2006; Bunce, 2008; Flexas *et al.*, 2008; Warren, 2008a). The K_{leaf} also increases in response to temperature, both due to the increase in viscosity of water and to the greater permeability of membranes in the outside-xylem pathways (Sack *et al.*, 2004). Few studies have analysed the short-term dynamics of g_m in response to irradiance, yet most of them except Tazoe *et al.* (2009) have found a positive correlation (Gorton *et al.*, 2003; Flexas *et al.*, 2007; Douthe *et al.*, 2011), and g_m seems to be modulated by light quality as well (Loreto *et al.*, 2009); further, shade leaves have lower g_m than sun leaves (Hanba *et al.*, 2002; Piel *et al.*, 2002; Warren *et al.*, 2007; Monti *et al.*, 2009). These responses are similar in direction to those of K_{leaf}, which in a number of species increases in the short-term with irradiance, and depends on light quality, and increases over the long term during acclimation to sun versus shade (e.g. Sack *et al.*, 2002, 2003; Tyree *et al.*, 2005; Cochard *et al.*, 2007; Scoffoni *et al.*, 2008; Brodribb and Jordan, 2011; Sellin *et al.*, 2011).

Of all environmental conditions, the influence of water stress (from short-term leaf dehydration to long-term drought or exposure to salinity) is by far the one for which most reports on the dynamics of g_m and K_{leaf} have been published. Regardless of the velocity of water stress imposition, g_m strongly decreases in response to water stress in a wide range of species (e.g. Bongi and Loreto, 1989; Roupsard *et al.*, 1996; Flexas *et al.*, 2002, 2006a, 2009; Diaz-Espejo *et al.*, 2007; Galmés *et al.*, 2007; Perez-Martin *et al.*, 2009; Misson *et al.*, 2010; Galle *et al.*, 2011). Similarly, the strong decline of K_{leaf} with dehydration has been studied in over 100 species (e.g. Nardini *et al.*, 2001; Brodribb and Holbrook, 2003; Scoffoni *et al.*, 2012). The bulk of studies show a strong decline of g_m with leaf dehydration; only a few studies have reported minor effects on g_m (e.g. Warren *et al.*, 2011). Similarly, the K_{leaf} declines with water stress in all species, but the steepness and shape of this decline varies across species, with more drought-sensitive species experiencing stronger declines at mild dehydration (Scoffoni *et al.*, 2012).

However, based on the data available, the responses of g_m to CO$_2$ and VPD do not parallel those of K_{leaf}. A large number of studies using different species and methods to estimate g_m have observed that low CO$_2$ increases g_m (Centritto *et al.*, 2003), and rapidly increasing CO$_2$ results in strong decreases of g_m (Düring, 2003; Flexas *et al.*, 2007; Hassiotou *et al.*,

Table 1. *Coordinated dynamics of leaf hydraulic and mesophyll conductance with short-term changes water status, irradiance, vapour pressure deficit (VPD), temperature, and* CO_2

	Resulting response of g_m	**Example references**	**Resulting response of K_{leaf}**	**Example references**
Declining water status	Decrease, 0–100%	Roupsard *et al.* (1996); Flexas *et al.* (2002); Galmés *et al.* (2007); Perez-Martin *et al.* (2009); Misson *et al.* (2010); Galle *et al.* (2011)	Decrease, up to 100%	Nardini *et al.* (2001); Brodribb and Holbrook (2003); Blackman *et al.* (2010); Scoffoni *et al.* (2012)
Increasing light	Increase, 0–250%	Gorton *et al.* (2003); Flexas *et al.* (2007); Tazoe *et al.* (2009); Douthe *et al* (2011)	Increase, 0–367%	Sack *et al.* (2002); Tyree *et al.* (2005); Cochard *et al.* (2007); Scoffoni *et al.* (2008)
Increasing VPD	Decrease, 0–60%	Bongi and Loreto (1989); Warren (2008), Pérez-Martín *et al*. (2009)	Increase, up to 401%	Levin *et al.* (2007)
Increasing temperature	Increase, 0–400%[a]	Bernacchi *et al.* (2002); Warren and Dreyer (2006); Yamori *et al.* (2006); Warren (2007); Flexas *et al.* (2008); Evans and von Caemmerer (2013)	Increase, up to 175%	Sack *et al.* (2004)
Increasing CO_2	Decrease, 0–90%	Loreto *et al.* (1992); Singsaas *et al.* (2003); Flexas *et al*. (2007), Hassiotou *et al.* (2009); Tazoe *et al.* (2009); Vrábl *et al*. (2009); Bunce (2010); Douthe *et al.* (2011)	=	Bunce (2006)

[a] The g_m scaled with temperature with a slope of 5% per degree Celsius (Evans and von Caemmerer, 2013).

2009; Vrábl *et al.*, 2009; Yin *et al.*, 2009; Bunce, 2010; Douthe *et al.*, 2011). Only one study in wheat showed no response of g_m to increased CO_2 in the short term (Tazoe *et al.*, 2009). In the long term, Singsaas *et al.* (2003) reported decreased g_m, with higher CO_2 only in some species and not in many others. Velikova *et al.* (2009) showed in *Platanus orientalis* that new leaves emerged when plants were already growing at high CO_2 showed much larger decreases of g_m than pre-existing leaves. Salazar-Parra *et al.* (2012) also showed decreased g_m with long-term high CO_2 in grapevines. By contrast, recent studies have found no effect of high CO_2 on K_{leaf} in soybean (Bunce, 2006; Locke *et al.*, 2013). More studies will be needed to confirm such a lack of response in K_{leaf} to CO_2.

The response of g_m to VPD is less certain. Under a sudden increase in VPD, Bongi and Loreto (1989) observed a decreased g_m in *Olea europaea*, whereas Warren (2008b) did not observe any response in several species, including *Eucalyptus regnans*, *Phaseolus vulgaris*, and *Solanum lycopersicum*. Perez-Martin *et al.* (2009) observed longer-term acclimation of g_m to VPD in *Olea europaea* and *Vitis vinifera*. One study has shown an increase in K_{leaf} in response to VPD (Levin *et al.*, 2007).

Thus, the similar responses of g_m and K_{leaf} to several environmental factors—notably, temperature, light, and leaf water status—provide another line of evidence for their mechanistic coordination and integrated influence on leaf function under dynamic conditions.

Mechanistic basis for the coordination: the outside-xylem tissues

Previous work on the coordination of K_{leaf} with photosynthetic rate per leaf area (A_{area}) has emphasized the influence of K_{leaf} on stomatal conductance (g_s), mediated by leaf water potential (Sack and Holbrook, 2006; Holloway-Phillips and Brodribb, 2011). However, this work's novel finding of a coordination of g_m and K_{leaf} in the compiled data, in which g_s was uncorrelated with K_{leaf}, implies that the coordination of A_{area} with K_{leaf} is due to more than simply the scaling of hydraulic and stomatal conductances. It is posited that properties of the outside xylem pathways which influence K_{leaf} and g_m in common underlie this coordination (see network diagram in Fig. 4). Based on a previously developed conceptual model for the coordination of leaf traits (Sack and Scoffoni, 2013a; Sack *et al.*, 2013) synthesized from previous studies of a wide range of species sets, the common basis of K_{leaf} and g_m can be clarified. In brief, K_{leaf} and its components, the vein xylem hydraulic conductance (K_x) and the outside-xylem hydraulic conductance (K_{ox}) are influenced by a number of leaf vein traits (Cochard *et al.*, 2004; Sack *et al.*, 2004, 2005; McKown *et al.*, 2010; Sack and Scoffoni, 2013b):

$$K_{leaf} = \left(K_x{}^{-1} + K_{ox}{}^{-1}\right)^{-1} \quad (1)$$

The K_x is influenced by the vein cross-sectional conductivity of each vein order, which is in turn influenced by the numbers and dimensions of the xylem cells. Further, K_x and K_{ox} depend on the *VLA* and the number and size of free-ending veins (FEVs), and the sizes, numbers, and permeability of the bundle sheath and bundle sheath extensions (BS and BSEs) along with the mesophyll water flow pathways (MPs). Additional vein traits influence the sensitivity of K_x to xylem embolism: the major vein length per area (major *VLA*) and the topology of the vein system. These aspects of the vein

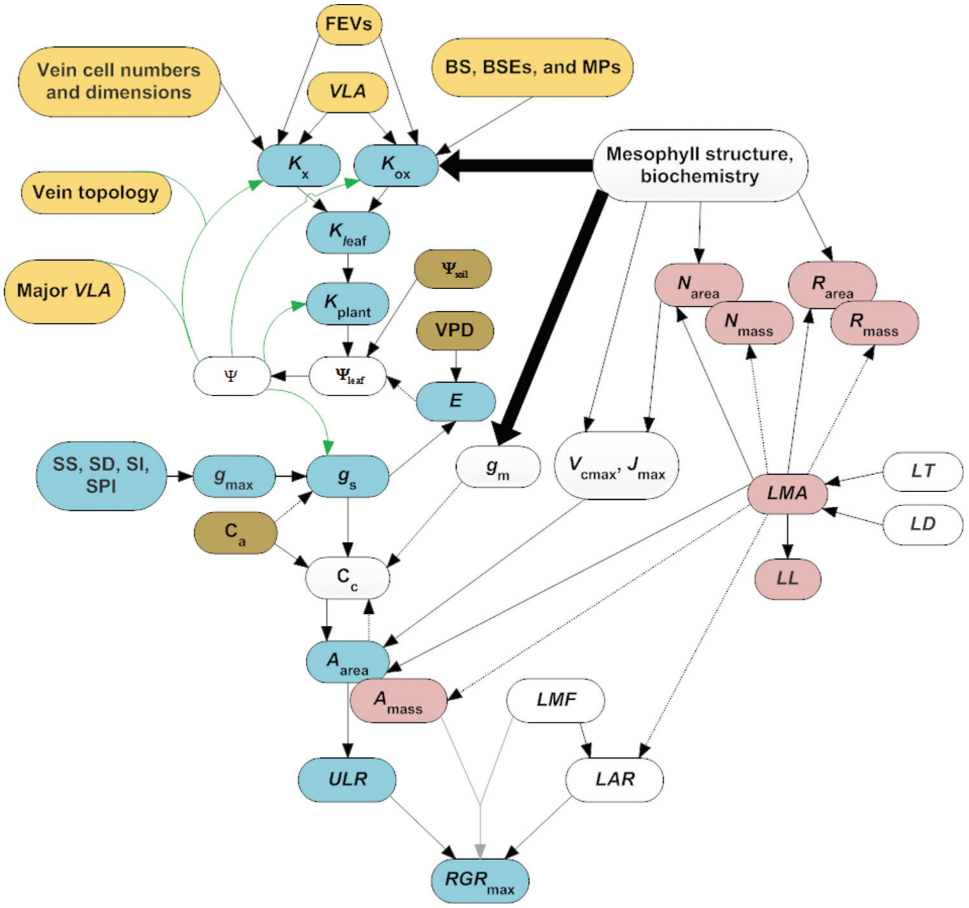

Fig. 4. The coordinated influence of leaf traits (including vein traits) on hydraulic, stomatal, and photosynthetic traits and whole-plant relative growth rate, based on a synthesis of the previous literature (updated from Sack and Scoffoni, 2013a and Sack *et al.*, 2013; see text for definitions and explanation). Yellow-shaded variables are vein traits; brown variables are related to soil, climate, and atmosphere; blue variables are flux-related traits that have been found or hypothesized to be correlated across species and to be linked with vein traits; pink variables are other leaf economics variables; and white variables are additional important traits. For several traits, both leaf area-based expressions (such as A_{area}, N_{area}, and R_{area}) and leaf mass-based expressions (A_{mass}, N_{mass}, and R_{mass}) are shown. Note that the 'Ψ' represents the water potential of different tissues in the plant, correlated with leaf water potential (Ψ_{leaf}) which drives impacts on hydraulic conductances and stomatal conductance. Black solid arrows signify positive mechanistic linkages; black dotted arrows indicate negative mechanistic linkages; green arrows indicate dynamics of hydraulic and stomatal conductances with water status; grey arrows indicate an alternative way to consider the impact of physiological traits on RGR_{max} (i.e. showing the more direct influence of A_{mass} on RGR_{max}, than that of A_{area}). Other possible impacts of *VLA* on A_{area} are not shown, including effects via improving sugar and nutrient transport. This schema is simplified, emphasizing the influence of venation on higher-level traits, and many other influences of traits on other traits are not shown (e.g. the negative influence of *ULR* and RGR_{max} by R_{area} and R_{mass}). The emboldened arrows highlight the partial common basis for the outside-xylem hydraulic conductance (K_{ox}) which influences K_{leaf}, and the mesophyll conductance to CO_2 diffusion (g_m).

system influence the abundance of redundant high conductivity pathways around embolized xylem conduits.

Because the leaf is an important hydraulic limitation in the plant, and K_{leaf} scales with the whole plant hydraulic conductance (K_{plant}) (Sack *et al.*, 2003; Sack and Holbrook, 2006), vein traits scale up to influencing K_{plant}, by the Ohm's law analogy, leaf water potential (Ψ_{leaf}) at a given transpiration rate (E):

$$\Delta\Psi_{leaf-to-soil} = E / K_{plant} \tag{2}$$

$$\Psi_{leaf} = E / K_{plant} + \Psi_{soil} \tag{2a}$$

where $\Delta\Psi_{leaf\text{-}to\text{-}soil}$ is the water potential gradient between leaf and soil, and Ψ_{soil} is the soil water potential. Coordination with stomatal conductance arises because

$$E = g \times \mathrm{VPD} \tag{3}$$

where VPD is the vapour pressure deficit, and g is leaf conductance to water vapour, primarily driven by the stomatal conductance (g_s) under wind of sufficient speed that the the boundary layer conductance (g_b) is not limiting,

$$\Psi_{leaf} = (g_s \times \mathrm{VPD}) / K_{plant} + \Psi_{soil} \tag{4}$$

Given these relationships, in many cases across species, g_s must be coordinated with K_{plant} (i.e. matching hydraulic demand with supply; Tyree and Zimmermann, 2002; Sack et al., 2005; Sack and Holbrook, 2006). That would be the case when Ψ_{leaf} varies only within narrow limits at a given range of VPD and Ψ_{soil}. Howver, for plants under substantially different soil moisture or VPD regimes, the coordination of g_s and K_{plant} may shift (a point to be further discussed). For a given species, the g_s is a function of the maximum stomatal conductance (g_{max}) and the degree that stomatal closure declines as the leaf dehydrates to a given Ψ_{leaf}. The g_{max} is a function of stomatal dimensions and numbers (quantified as stomatal size, density, index, and pore area index; Sack et al., 2003; Franks and Farquhar, 2007).

Ultimately, the photosynthetic rate per leaf area (A_{area}) is importantly controlled by this hydraulic-stomatal coordination. The A_{area} is determined by the chloroplastic CO_2 concentration (C_c) and biochemical parameters including the electron transport rate (J_{max}) and the maximum rate of carboxylation (V_{cmax}), while C_c is depends on the ambient CO_2 concentration (C_a), g_s, and mesophyll conductance (g_m; Farquhar et al., 2001; Flexas et al., 2012b). The J_{max} and V_{cmax} depend on the activity of light and carbon reactions per leaf area, and would relate to leaf nitrogen concentration per area (N_{area}), as would the respiration rate (R_{area}) (Evans et al., 2000).

The K_x and K_{ox} are dynamic, like K_{leaf}, and the hydraulic conductances elsewhere in the plant. All are sensitive to declining water potentials (Ψ) throughout the plant system, resulting from embolism in roots, stems, or leaves, tissue collapse, and/or biochemical changes. Further, declining water potential in or around the guard cells drive g_s decline (Hubbard et al., 2001; Guyot et al., 2012; Scoffoni et al., 2012). The susceptibilities of different tissues will thus scale up to impacting on K_{plant} and gas exchange.

Other leaf traits also influence higher-level function. The *LMA* (which itself is equal to the product of leaf thickness and density; LT and LD respectively), is typically negatively correlated with mass-based nitrogen concentration and rates of respiration and photosynthesis (N_{mass}, R_{mass}, and A_{mass}, respectively) (see previous discussion). A higher *LMA* typically corresponds with a greater leaf lifespan (LL; Wright et al., 2004).

Vein traits can thus influence whole-plant maximum relative growth rate (RGR_{max}) (Evans, 1972; Poorter et al., 2009). The A_{area} is the major driver of the dry mass accumulation per leaf area (unit leaf rate, *ULR*). The *RGR* is determined as the product of *ULR* and the leaf area ratio (*LAR* =leaf area/plant mass); the *LAR* itself is equal to the leaf mass fraction (*LMF*=leaf mass/plant mass) divided by *LMA* plant maximum relative growth rate (RGR_{max}) (Evans, 1972; Poorter et al., 2009).

The framework we have described for the potential relationships among vein traits, hydraulic traits and gas exchange traits is expected to arise clearly for some but not for all species sets. As explained above, the correlations among traits that would emerge due to equation 4 will do so only when species vary narrowly in their operating Ψ_{leaf}, Ψ_{soil}, and VPD. Thus, the particular coordination among traits in this framework will differ according to the habitat or environment of the species compared (Sack et al., 2005, 2013; Feild et al., 2011). Additionally, when traits are determined by two or more traits, the importance of those traits will depend on the species set. For example, because A_{area} depends on g_s and g_m, and both are potentially coordinated with K_{leaf}, but differently depending on the species and its habitat, the coordination of A_{area} with g_m and with venation traits will differ among species sets, as expected according to the framework relating vein traits to gas exchange and whole-plant growth. Recent work has also indicated that higher *VLA* can also enable an increased phloem transport capacity that can also drive higher A_{area} (Fu et al., 2011; Nikinmaa et al., 2012; Sack and Scoffoni, 2013b). The A_{area}, may also be influenced by nutrient delivery rates, which also may depend on vein traits (Shabala et al., 2002; Kerton et al., 2009; Gilliham et al., 2011), even in mature leaves which need to replace degraded proteins and pigments (Girardin et al., 1985; Niinemets et al., 2004).

Based on the coordination observed between g_m, K_{leaf}, and A_{area} across diverse species and their partial similarity in responses to environmental conditions, this work hypothesizes that mesophyll structural and physiological traits that affect g_m and photosynthetic processes also have an influence on leaf hydraulic capacity. In principle, both K_{ox} and g_m may depend on aquaporin activity (Ferrio et al., 2012). The mechanisms for coordinated dynamics of g_m and K_{leaf} remain to be clarified (Flexas et al., 2012b; Griffiths and Helliker, 2013). Further, the partially distinct dynamics of these traits (e.g. CO_2 response of g_m but not of K_{leaf}) are consistent with the partially independent pathways for the movement of water and CO_2 inside leaves. For instance, it is well known that water moves in leaves through xylem conduits within the veins and, upon exiting the bundle sheath, water moves through the mesophyll via apoplastic, symplastic, and/or cell-to-cell pathways (Sack and Holbrook, 2006). The g_m might be related only to the non-xylem component of K_{leaf}. The question of whether CO_2 importantly moves through apoplastic, symplastic, or transmembrane cell-to-cell pathways has never been addressed, but it is probable that, in general, for C_3 plants, CO_2 diffuses simply from intercellular air spaces directly into each photosynthesizing mesophyll cell which acts as a final sink. If so, the 'symplastic' pathway for CO_2 does not exist, and then the 'apoplastic' and 'transmembrane' pathways are components of the diffusion into single cells. Thus, a CO_2 molecule from the intracellular air spaces has to first pass the apoplast/cell wall then across the cell membrane, the cytosol, and the chloroplast outer membrane to reach the site of carboxylation in the chloroplast matrix. These components are all potentially quantitatively important. In fact, cell-wall thickness has been found to represent a strong limitation on g_m (Peguero-Pina et al., 2012; Scafaro et al., 2011; Tosens et al., 2012; Tomás et al., 2013). Further, membrane aquaporins—which are known to facilitate transmembrane water but also CO_2 transport (Tyerman et al., 2002; Kaldenhoff et al., 2008)—also induce modifications of g_m (Hanba et al., 2004; Flexas et al., 2006b; Uehlein et al., 2008; Heckwolf et al., 2011).

Thus, g_m and K_{ox} are both likely to be influenced in common by cell-wall and membrane properties that would affect the apoplastic and transmembrane pathways, respectively. Such overlap in mechanistic basis would explain the correlation between g_m and K_{leaf} (Fig. 1) and their parallel dynamics in response to changes in irradiance and water supply which might affect a wide range of leaf cells and/or aquaporins. However, different mechanisms operating at a range of scales would independently regulate g_m and K_{leaf} in response to dynamic environmental changes, accounting for their divergent responses (e.g. the decrease of g_m in response to sudden CO_2 increase, while K_{leaf} is unaffected). A recent study has demonstrated rapid changes in mesophyll aquaporin gene expression in response to CO_2 (Francesca Secchi and Claudio Lovisolo, personal communication). However, K_{ox}, and thus maybe K_{leaf} as a whole, may not be influenced by the permeability of mesophyll cells, but rather by that of vein and bundle sheath cells (Prado *et al.*, 2013). Further, even if aquaporins respond in cells that influence both g_m and K_{ox}, the two variables may respond differently to aquaporin activity. Otto *et al.* (2010) have shown a trade off between water and CO_2 permeability through membranes, depending on the proportion of PIP1 and PIP2 aquaporins in the tetramer aquaporin assembly in membranes. Therefore, even if CO_2 affects aquaporin synthesis and assembly, resulting in a configuration which reduces g_m, K_{leaf} may be unaffected or even increased.

Isotopic studies also point to the fact that g_m and K_{leaf} share a partial basis, particularly in aquaporin-dependent pathways. Thus, studies in which g_m was measured alongside isotope discrimination to ^{18}O allow comparisons of g_m with an estimate of 'scaled effective path length' (L_{eff}) for water transport from xylem vessels to the sites of evaporation. The latter parameter is strongly and inversely correlated to K_{leaf}, and especially to K_{ox}, as shown in grapevines (Ferrio *et al.*, 2012). The L_{eff} was also strongly correlated with g_m in grapevines subjected to either irrigation or water stress and with the main leaf veins intact or cut (Ferrio *et al.*, 2012). Using the same approach, Flexas *et al.* (2012b) demonstrated that L_{eff} and g_m were also correlated in transgenic tobacco plants with g_m altered by modifying aquaporin expression.

Can empirical relationships be used to estimate g_m from K_{leaf} or vice-versa?

The strong coordination between g_m and K_{leaf} suggests that it may be possible to use knowledge of one of these parameters to at least roughly estimate the other. Measurement of K_{leaf} and g_m is rewarding but complex and time-consuming. Indeed values for both parameters were found for only 23 species in the compiled database (Supplementary Table S1). However, having data for these parameters for a wide range of species is crucial for whole-plant and ecosystem models. For instance, the time-response courses of CO_2 assimilation and its sensitivity to environmental change scenarios can be better modelled if dynamics of g_m and K_{leaf} are available, but the lack of information for many species precludes their use (Niinemets *et al.*, 2009, 2011). The possibility of retrieving g_m values from measured K_{leaf} or vice versa would result in a fast expansion of the number of species for which these leaf traits are available.

On the other hand, the complex trait network in which both K_{leaf} and g_m are inter-related (Fig. 4) suggests that simple two parameter relationships as those shown in Fig. 1 may not capture the true co-determination among these two parameters and others. Biological processes are typically highly complex, dynamic and non-linear. Thus, artificial intelligence technology can be a useful mathematical approach to decipher and identify complex non-linear interactions between parameters controlling a process (Prasad and Dutta Gupta, 2008; Gago *et al.*, 2010). Here, to develop predictive equations, the current employed neurofuzzy logic technology, a hybrid technology based on artificial neural networks (ANNs) combined with fuzzy logic, to retrieve and integrate knowledge hidden within the compiled dataset for the 17 species with known values for K_{leaf}, *LMA*, and $g_{m.}$ This technology was described previously as a useful data mining tool to promote the understanding of complex processes in plant science (Gago *et al.*, 2011). The current work used FormRules version 3.31 (2008; Intelligensys, UK), a commercial neurofuzzy logic software, implementing the ASMOD (Adaptative Spline MODeling of data) algorithm (Kavli and Weyer, 1994). This methodology generates models that are sums or products of the variables into smaller submodels that depend only on a subset of inputs using a global partitioning technique (Gago *et al.*, 2011). A neurofuzzy logic submodel was successfully developed for the parameter (output) g_m as a function of two variables (inputs): *LMA* and K_{leaf}. The inputs had independent effects on g_m. The work found a strong correlation between the experimentally measured and the predicted g_m based on neurofuzzy logic analysis ($R^{2=}0.64$, $P<0.05$; Fig. 5A). The g_m predicted by the model as a function of both *LMA* and K_{leaf} are presented in a 3-D plot (Fig. 5B). Notably, increased K_{leaf} was associated with slight increases in g_m, and the highest g_m values were achieved at lower *LMA* values; the highest values of g_m were observed when K_{leaf} was high (>14.5 mmol m^{-2} s^{-1} MPa) and *LMA* was low (<115 g m^{-2}). The observed relationship suggests that it may be possible to estimate g_m from K_{leaf} and other simple parameters such as *LMA* or A_{area}, or, conversely, to estimate K_{leaf} from a complete gas exchange analysis. It is important to bear in mind that the strength of ANNs arises from an ability to model complex non-linear relationships between inputs and outputs; however, the model yielded is a 'black-box', and interpretation is not possible, depending on other technologies such as fuzzy logic, genetic algorigthms, or GEP (Genetic Expression Programming) (Gallego *et al.*, 2011). In this sense, an exciting and promising research frontier is the use of ANNs coupled to GEP technology to develop predictive empirical equations relating hydraulics and mesophyll conductance; unfortunately, due to the current paucity of data for both parameters a validated empirical equation could not be generated with this technology. The neurofuzzy logic training parameters and software information can be seen in Supplementary Table S2. Also, in an attempt to retrieve an empirical equation, a multivariate regression analysis was performed on the same dataset (using R statistics software). A polynomial equation, with g_m as the

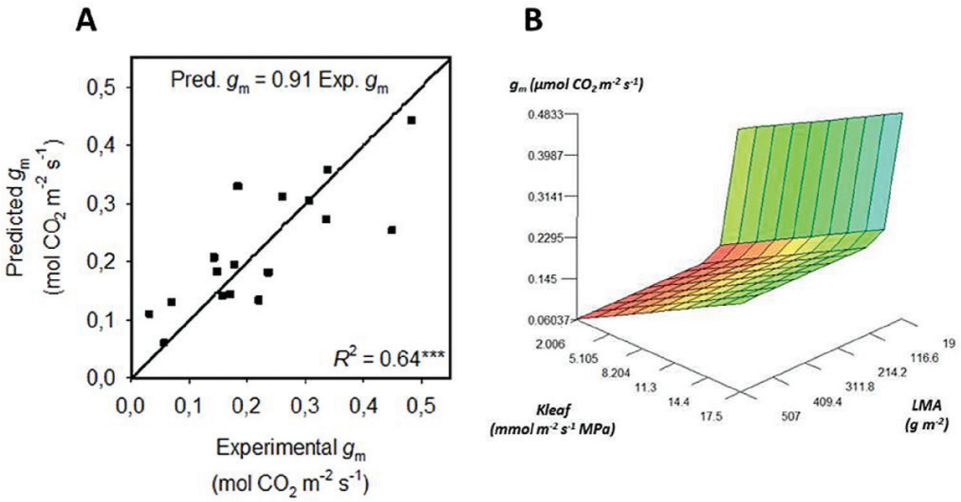

Fig. 5. (A) The relationship between measured g_m and that predicted by a model based on neurofuzzy logics analysis. Lines were forced through the origin so that the slope deviation from 1.0 represents the bias of the prediction. The presented R^2 are for lines not forced through the origin. The model was fed by data for K_{leaf} and *LMA* based on the 17 species in a compiled database in which g_m, K_{leaf}, and *LMA* were available (Supplementary Table S1). (B) The 3-D plot showing the interdependency among the three parameters (this figure is available in colour at *JXB* online).

dependent variable and K_{leaf}, *LMA*, and *VLA* as independent variables was fitted by stepwise multiple regressions. Similar results were obtained as with the neurofuzzy logic technology, although with a weaker predictive power ($R^{2=}0.43$, $P<0.05$). The following equation was obtained:

$$g_m = 0.293 - 0.001LMA + 0.009K_{leaf} - 0.010VLA$$

Studies in which g_m, K_{leaf}, and *LMA* are simultaneously determined are important to confirm and to obtain a truly predictive model or equation for these parameters (Fig. 5).

Outstanding research possibilities emerging from the combined determination of g_m and K_{leaf}

A new understanding of leaf physiology includes required knowledge of all transport processes, for water and CO_2, and the structures involved, i.e. from veins to mesophyll cells. The new generation of whole-plant physiologists will have combined skills for advanced measurements of leaf gas exchange and hydraulic function, and the ability to interpret and utilize data of both types. Knowledge of the combined responses will enable a new ability to model the entire system, in turn allowing better predictions of gas exchange and its dynamics, and thus of the influence of environmental factors on photosynthetic rate and plant growth. A detailed elucidation of the common basis of g_m and K_{leaf} (e.g. the shared aspect of the transport pathways and the role of aquaporins) can provide a clearer ability to scale up from molecules to whole leaf function, and potentially, via crop breeding, to influence both aspects of leaf function in parallel, with a synergistic influence on the integrated hydraulic-photosynthetic system.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Table S1. Multi-species dataset compilation.

Supplementary Table S2. Training parameters setting for FormRules version 3.31.

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