Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation

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Abstract

Foliage structure, chemistry, photosynthetic potentials (Vcmax and Jmax), and mesophyll diffusion conductance (gm) were quantified for 35 broad-leaved species from four sites with contrasting rainfall and soil fertility in eastern Australia. The aim of the study was to estimate the extent to which gm and related leaf properties limited photosynthesis (A), focusing on highly sclerophyllous species typical of the ‘slow-return’ end of the leaf economics spectrum. Leaf dry mass per unit area (MA) varied ~5-fold, leaf life span (Ll) and N (NM) and P (PM) contents per dry mass ~8-fold, and various characteristics of foliage photosynthetic machinery 6- to 12-fold across the data set. As is characteristic of the ‘leaf economics spectrum’, more robust leaves with greater MA and longevity were associated with lower nutrient contents and lower foliage photosynthetic potentials. gm was positively correlated with Vcmax and Jmax, and these correlations were stronger on a mass basis. Only gm/mass was negatively associated with MA. CO2 drawdown from substomatal cavities to chloroplasts (C5–C3) characterizing mesophyll CO2 diffusion limitations was larger in leaves with greater MA, lower gm/mass, and lower photosynthetic potentials. Relative limitation of A due to finite mesophyll diffusion conductance, i.e. 1–A(infinite gm)/A(actual gm), was always >0.2 and up to 0.5 in leaves with most robust leaf structure, demonstrating the profound effect of finite gm on realized photosynthesis rates. Data from different sites were overlapping in bivariate relationships, and the variability of average values between the sites was less than among the species within the sites. Nevertheless, photosynthesis was more strongly limited by gm in low rain/high nutrient and high rain/low nutrient sites that supported vegetation with more sclerophyllous foliage. These data collectively highlight a strong relationship between leaf structure and gm, and demonstrate that realized photosynthesis rates are strongly limited by gm in this highly sclerophyllous flora.

Key words: Assimilation rates, diffusion limitations, foliage structure, limited nutrients, nitrogen content, phosphorus content, sclerophylls, structure–function relationships, water availability.

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Abbreviations: A (µmol m⁻² s⁻¹), net assimilation rate; Amax (µmol m⁻² s⁻¹), hypothetical A in the absence of gₘ; A₇ (µmol m⁻² s⁻¹), A standardized to Cₛ = 250 µmol mol⁻¹; Cₛ (µmol mol⁻¹), ambient CO2 concentration; Cₐ (µmol mol⁻¹), CO2 concentration in chloroplasts; Cₐ/Cₑ (µmol mol⁻¹), Cₐ standardized to Cₛ=250 µmol mol⁻¹; Cₐ (µmol mol⁻¹), CO2 concentration in substomatal cavities; Dₛ (g g⁻¹), leaf dry to fresh mass ratio; gₘ, mesophyll diffusion conductance to CO₂; gₘ/area (mol m⁻² s⁻¹); gₘ per unit area; gₘ/area (µmol g⁻¹ s⁻¹); gₘ per unit dry mass; Jₑ,TR, rate of photosynthetic electron transport from fluorescence (Eq. 1); Jₑmax, capacity for photosynthetic electron transport; Jₑmax (µmol m⁻² s⁻¹), Jₑmax per unit area; Jₑmax/mass (µmol g⁻¹ s⁻¹), Jₑmax per unit dry mass; Lₗ (year), leaf life span; Mₐ (g m⁻²), leaf dry mass per unit area; Nₐ (g m⁻²), leaf nitrogen content per unit area; Nₐ (%) leaf nitrogen content per dry mass; Pₛ (g m⁻²), leaf phosphorus content per area; Pₛ (%) leaf phosphorus content per dry mass; Qₑ (µmol m⁻² s⁻¹), non-photosynthetic respiration rate continuing in light; T (µm), leaf thickness; Vₑmax, maximum carboxylase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco); Vₑmax/area (µmol m⁻² s⁻¹); Vₑmax per unit area; Vₑmax/mass (µmol g⁻¹ s⁻¹), Vₑmax per unit dry mass; Φₑtot (mol mol⁻¹), effective quantum yield of photosystem II; Aₛ, relative limitation of photosynthesis due to gₘ (Eq. 3).

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Introduction

During photosynthesis, CO₂ diffusion from substomatal cavities to chloroplasts is limited by mesophyll diffusion conductance \( (g_m) \) that reduces the CO₂ concentration in chloroplasts \( (C_C) \) relative to that in substomatal cavities \( (C_i) \). There has been increasing recognition that \( g_m \) varies considerably among species; about two orders of magnitude from \( \sim20\text{ mmol m}^{-2}\text{s}^{-1} \) to \( 2000\text{ mmol m}^{-2}\text{s}^{-1} \) (Terashima et al., 2006; Flexas et al., 2008; Warren, 2008; Niinemets et al., 2009). Mesophyll diffusion limitations have traditionally been considered to be especially large in structurally robust leaves, with high values of leaf dry mass per unit area \( (M_A) \) (Nobel, 1977). Until recently, it was commonly assumed that interspecific variation in \( C_i\) was small enough to be neglected. Indeed, \( g_m \) is often assumed to be infinite and \( C_C \) is set to \( C_i \) in application of the most widely employed biochemical photosynthesis model (Farquhar et al., 1980) to simulate plant carbon gain at leaf, stand, and biome levels (Dai et al., 2004; Medlyn, 2004; Hickler et al., 2008).

By definition, the rate of net assimilation, \( A \), is associated with \( g_m \) according to \( A = g_m(C_i - C_C) \). Thus, the magnitude of CO₂ drawdown, \( C_i - C_C = A/g_m \), depends on both \( g_m \) and \( A \). In assessing the impact of a given value of \( g_m \) on diffusional limitations of photosynthesis, it is therefore imperative to examine the CO₂ drawdowns corresponding to specific \( g_m \) values. The positive correlations observed between \( g_m \) and foliage photosynthetic capacity have led to alternative views on whether the impact of \( g_m \) on realized photosynthetic rates is influenced by leaf structure (Evans and Loreto, 2000; Flexas et al., 2008). Such positive correlations have been interpreted as indicative of constant CO₂ drawdown \( (A/g_m) \) despite variations in \( g_m \) among species with contrasting foliage structure (Evans and Loreto, 2000). However, recent meta-analyses have demonstrated that \( C_i - C_C \) does scale negatively with \( g_m \), and interspecific variation in \( g_m \) is associated with large variation in CO₂ drawdown (Niinemets et al., 2005a; 2009; Niinemets and Sack, 2006; Warren and Adams, 2006; Warren, 2008). In fact, negative correlations between \( g_m \) and \( M_A \) (Terashima et al., 2005; Flexas et al., 2008) and positive correlations between \( M_A \) and \( C_i - C_C \) have been reported (Niinemets et al., 2005a; Niinemets and Sack, 2006), suggesting that photosynthesis is more strongly limited by mesophyll diffusion in structurally more robust leaves. However, so far \( g_m \) has been studied in only \( \sim120 \) species, and, for these species, \( M_A \) versus \( g_m \) and \( C_i - C_C \) versus \( g_m \) relationships are characterized by large variability that is not understood (Flexas et al., 2008; Warren, 2008). It has been argued that because \( C_i - C_C \) is a mesophyll volume-weighted average, not a leaf area-weighted average, \( C_i - C_C \) should better scale with \( g_m/\text{mass} \) than with the more traditionally used \( g_m/\text{area} \) (Niinemets et al., 2005a; Niinemets and Sack, 2006), but \( g_m/\text{mass} \) versus \( g_m/\text{area} \) relationships have not been studied for a large set of structurally varying leaves.

Apart from the limited knowledge of structural controls on \( g_m \), there is lack of information of \( g_m \) for sclerophyllous species with \( M_A \) values larger than \( \sim180\text{ g m}^{-2} \) (Flexas et al., 2008), making the assessment of the worldwide role of \( g_m \) in plant carbon gain currently not feasible. In addition, there are no comprehensive studies of \( g_m \) variation among co-existing species of a given ecosystem, and potential ecosystem-level differences in \( g_m \) limitation of photosynthesis have not been assessed.

To gain more advanced insight into the structural controls of \( g_m \) and fill the gaps in the available data, \( g_m \) was analysed in relation to foliar photosynthetic potentials and foliar structure in 35 eastern Australian species exhibiting extremely low foliage N and P contents and especially high values of \( M_A \). Australian ecosystems on old highly leached soils are characterized by exceptionally low soil P and N availabilities (Specht, 1969; di Castri, 1981) and support highly sclerophyllous vegetation (Hill, 1998). In a worldwide context, Australian broad-leaved sclerophylls are positioned in the lower nutrient/higher \( M_A \) and leaf longevity end of the plant functional spectrum (Wright and Westoby, 2003; Wright et al., 2004a; Denton et al., 2007). It was suggested that these sclerophylls belong worldwide to the species groups with the largest CO₂ drawdowns due to low \( g_m \). With this exceptional data set, the following hypotheses were tested: (i) \( g_m \) is negatively correlated with \( M_A \), and thus with leaf life span; and (ii) the CO₂ drawdown, \( C_i - C_C \), characterizing the limitation of photosynthesis by \( g_m \) is larger in species with more robust foliage (higher \( M_A \) and associated traits) and lower photosynthetic potentials, and smaller in species with higher \( g_m \), i.e. the interspecific variation in CO₂ drawdown is driven by species variation in foliage structure. It was further suggested that (iii) the correlations of \( g_m \) with foliage photosynthetic potentials, \( M_A \), and \( C_i - C_C \) are stronger for \( g_m/\text{mass} \) than for \( g_m/\text{area} \). The study was accomplished in four sites, chosen as high/low fertility sites (clay-rich or sandy) within each of two rainfall zones (high/low). Although all sites had low nutrient availabilities considered in a global context, it was suggested that (iv) photosynthesis is on average more limited by \( g_m \), in more stressful low rain/lower nutrient sites where foliage is on average more sclerophyllous than in high rain/higher nutrient sites (Wright and Westoby, 1999).

There is evidence that in leaves with a given structure, \( g_m \) may change relatively rapidly in response to variation in environmental variables and in response to stress for reasons not yet fully known (Flexas et al., 2008). Here the focus is on maximum steady-state values of \( g_m \) at current ambient CO₂ concentrations without such interfering environmental effects.

Materials and methods

Study sites and species selection

The study was conducted in April 2006 in natural temperate broad-leaved evergreen ecosystems in the broad vicinity of Sydney, New South Wales, Australia (Table 1). A gradient of decreasing rainfall extends inland from the coast in this
region. The two higher rainfall sites in Ku-ring-gai Chase National Park receive annually ~1.5-fold, and during the driest period between August and October ~1.8-fold, less precipitation than the two sites, located ~50 km west of Sydney in the Cumberland Plain (Castlereagh Nature Reserve and Agnes Banks Nature Reserve; Table 1). In both wetter and drier site pairs, the community on clay-rich soil had higher soil nutrient availabilities than that occurring on deep sands (Table 1), with the drier/lower nutrient availability site (Agnes Banks) representing the extreme lowest values in soil nutrients, and the wetter/high nutrient site (West Head) the highest values (Table 1). At higher rainfall, the more fertile site supported closed forest with a rich understorey of ferns, cycads, shrubs, climbers, and herbs, while open woodland with species-rich heathy understorey occurs on the less fertile sand. The lower rainfall sites both supported open woodlands, with significant fractions of bare ground. A more detailed description of Ku-ring-gai Chase National Park sites is provided in Wright et al. (2001), while the drier sites are described in Benson (1992) and in NSW National Parks and Wildlife Service (1999).

The overall aim in species selection was to obtain a broad, representative range of foliage architectures, longevities, and photosynthetic potentials across the sites. In addition, in each site, species with contrasting leaf structure and life span were selected to characterize site-specific variation and site effects on average foliage traits. As the major constraint in species selection, only broad-leaved species suitable for gas-exchange measurements with clip-on gas-exchange cuvettes were sampled. [See Rodeghiero et al. (2007) for extensive discussion of problems in gas-exchange measurements in leaves that do not entirely fill the small cuvette window or that result in extensive air passage between the cuvette gaskets.]

At the more speciose, higher rainfall sites, 10 species were sampled at the more fertile low rainfall site and 13 species at the less fertile site. Six species were sampled at the more fertile low rainfall site, and three from the low nutrient site. To expand the overall variation range in foliage traits, naturally established individuals of three species (Pittosporum undulatum, Polyscias sambucifolia, and Acacia longifolia) and planted individuals of three other species (Banksia integrifolia, Banksia robusta, and Macadamia ternifolia) were sampled in the forest and parklands of the Macquarie University campus, North Ryde, Sydney (33°46′S, 151°06′E). The campus environment with annual average precipitation of 1132 mm, annual average maximum temperature of 22.8 °C and minimum temperature of 11.2 °C, and high nutrient availability most closely resembles the wetter/high nutrient availability site (Table 1). Neither foliage N (P > 0.13) nor foliage P (P > 0.5) contents per mass differed between campus and wetter/high nutrient site according to analysis of variance (ANOVA). Altogether 35 species were studied across all sites (see Appendix I for the species list with key foliage traits). Two of the studied species are gymnosperms (cycads: Macrozamia communis and M. spiralis); all others are angiosperms. Six species (Acacia falcata, A. longifolia, A. myrtifolia, A. suaveolens, Macrozamia communis, and M. spiralis) are nitrogen fixers.

### Plant sampling

Twig sampling and conditioning for gas-exchange measurements was conducted as detailed in Niinemets et al. (2005a). Exposed twigs of mature individuals were sampled in all cases in the morning hours when ambient air water vapour pressure was low. The selected twigs were cut under water, and again immediately recut under water. The cut end was maintained in water and the twigs were transported to the laboratory within an hour from the collection. In the laboratory, the twigs in water were covered with plastic
bags to reduce transpiration, and pre-conditioned at room temperature of 22 °C and at dim light of 50–100 μmol m⁻² s⁻¹ for 1–2 d before the gas-exchange measurements to stabilize stomatal conductance and reduce site-to-site and species-to-species differences in stomatal openness (Niinemets et al., 2005a).

At each site, 2–4 different plants of a given species were sampled, and on average (±SD) 2.51±0.82 individuals per species were investigated (altogether 88 combined gas-sampled, and on average (Niinemets et al., 2005a).

Gas-exchange and chlorophyll fluorescence measurements

A Li-Cor LI-6400 portable photosynthesis system with a LI-6400-40 Leaf Chamber Fluorometer (Li-Cor, Inc., Lincoln, NE, USA) was employed to measure net assimilation rate (A) versus internal (CO₂) concentration in substomatal cavities, C𝑖, CO₂ response curves simultaneously with the effective quantum yields of photosystem II following the protocol of Niinemets et al. (2005a). A saturating quantum flux density of 1000 μmol m⁻² s⁻¹ (20% blue LED, 80% red LED) provided by the leaf chamber fluorometer was used. Leaf temperature was maintained at 25 °C, and a leaf to air water vapour pressure deficit was kept <1.1 kPa. Fully mature current-season leaves were used in all cases. Whenever a single leaf did not fully fill up the window of the gas-exchange cuvette, several leaves arranged side by side were enclosed in the cuvette.

A low CO₂ of 50 μmol mol⁻¹ during the leaf stabilization period was used to ensure maximum stomatal openness before the measurement of A/𝐶𝑖 curves (Centritto et al., 2003). At this ambient CO₂ concentration, maximum stomatal conductance was achieved 20 min to 2 h after leaf enclosure in the cuvette. After reaching the maximum stomatal conductance, steady-state values of net assimilation rates were measured at eight ambient CO₂ concentrations between 50 μmol mol⁻¹ and 2000 μmol mol⁻¹. When the steady-state assimilation rate at a given CO₂ concentration, ~5 min after change in CO₂ concentration, had been recorded, the steady-state chlorophyll fluorescence yield in the light-adapted state, F, was estimated, and a single saturating pulse of white light of 6800–7000 μmol m⁻² s⁻¹ was applied to close all photosystem II centres and obtain the maximum fluorescence yield Fmax. From these measurements, the effective quantum yield of photosystem II, ΦPSII, was calculated as (Fmax–F)/Fmax. Upon completion of the A/𝐶𝑖 curve, the light was switched off, and as soon as the chamber temperature and ambient CO₂ concentrations had stabilized, commonly 1–2 min after leaf darkening, the dark respiration rate was recorded.

To determine the rates of photosynthetic electron transport from chlorophyll fluorescence measurements (JETR), leaf reflectance and transmittance were estimated with a Li-Cor portable spectroradiometer (Li-Cor 1800, Li-Cor, Inc.) equipped with a Taylor-type integrated sphere. These measurements along with the blue and red LED spectra of the LI-6400-40 Leaf Chamber Fluorometer light source (Li-Cor Inc., 2004) were used to estimate the amount of light absorbed, Qabs. JETR was determined as (Schreiber et al., 1994):

\[ J_{ETR} = 0.5\Phi_{PSII} Q_{abs} \]  

Determination of foliage gas-exchange characteristics

All foliage gas-exchange measurements were corrected for diffusion of CO₂ and water vapour through LI-6400 neoprene/polyethylene gaskets according to Rodeghiero et al. (2007). Mesophyll CO₂ diffusion conductance from the substomatal cavities to chloroplasts (gₘ) was calculated as (Harley et al., 1992):

\[ g_m = \frac{A}{C_i - \frac{J_{ETR} + 8(A + R_d)}{J_{ETR} - 4(A + R_d)}} \]  

where R_d is the non-photorespiratory respiration rate during the light period, and Γ* is the hypothetical CO₂ compensation point without R_d, Γ* = 42.9 μmol mol⁻¹ at 25 °C according to Bernacchi (2001). As previously (Niinemets et al., 2005a, 2006), R_d was taken as half of the rate of respiration measured in the dark. This simplification is supported by several experimental observations (Villar et al., 1995; Piel et al., 2002). Alternative estimation of gₘ by curve fitting according to Ethier et al. (2004) gave estimates of gₘ very similar to Eq. 2 (for a comparison between the two methods, see Niinemets et al., 2005a).

Average values of gₘ were computed for A values obtained for internal CO₂ concentrations of 150–350 μmol mol⁻¹. Over this C_i range, the values of gₘ are stable, and the estimates of gₘ are relatively insensitive to minor errors in Γ*, R_d, and A (Harley et al., 1992; Niinemets et al., 2006).

Chloroplastic CO₂ concentrations (C_C) for any given A were further calculated as C_C = A/gₘ, and the parameters of the photosynthesis model of Farquhar et al. (1980), the maximum carboxylase activity of Rubisco (Vmax), and the capacity for photosynthetic electron transport (I_max) were calculated as in Niinemets et al. (1999). In Vmax calculations, the values of Michaelis–Menten constants at 25 °C for CO₂ (K_C) of 274.8 μmol mol⁻¹ and for O₂ (K_o) of 414.1 mmol mol⁻¹ were from Bernacchi et al. (2001).

Characterizing the significance of differences in gₘ on photosynthesis

The impact of leaf-to-leaf differences in gₘ on photosynthesis depends on the drawdown of CO₂ from substomatal cavities to chloroplasts, i.e. the ratio A/gₘ. Actual average CO₂ drawdown from the substomatal cavities to chloroplasts, Cᵣ-C_C, and the ratio of C_C to Cᵣ were calculated for average Cᵣ and C_C values obtained for the ambient CO₂ concentration range of 320–420 μmol mol⁻¹ (average 380 μmol mol⁻¹). CO₂ drawdowns from ambient air (C_a) to chloroplasts (C_a-C_C) and from ambient air to substomatal cavities (C_a-Cᵣ) were also calculated.
To reduce the effect of leaf-to-leaf differences in stomatal openness that affect the CO₂ concentration in leaf substomatal cavities (Cᵢ), the fully parameterized model of Farquhar et al. (1980) was employed in an iterative manner to calculate the values of A and Cᵢ corresponding to a Cₒ of 250 μmol mol⁻¹ (Aᵢst and Cᵢst). From these simulated values, standardized estimates of Cᵢ/Cₒ and Cₑ/Cᵢ were derived. Overall, the average (±SE) Cᵢ corresponding to the ambient CO₂ range of 320–420 μmol mol⁻¹ was 246.8±4.5 μmol mol⁻¹ for all leaves measured, and Aᵢst and actual net assimilation rate were strongly correlated (r²=0.78, P < 0.001, n=88), as were the actual and standardized CO₂ drawdowns (r²=0.39, P < 0.001) and Cₑ/Cᵢ ratios (r²=0.49, P < 0.001). Both standardized and actual values resulted in similar statistical relationships with other foliage traits, but, in most cases, the standardized values gave somewhat larger degrees of explained variance (r²; see the Results).

To further characterize the diffusional limitations on photosynthesis, the model of Farquhar et al. (1980) was also used to determine the apparent rate of net assimilation (Aᵢapp) for a hypothetical situation of gₘ→∞, i.e. Cᵢ = Cᵢst. Cᵢst was assumed to be 250 μmol mol⁻¹ for all leaves, and Aᵢapp was calculated. The relative limitation of photosynthesis, AᵢD, due to limited mesophyll diffusion conductance was calculated as

\[ AᵢD = 1 - \frac{Aᵢst}{Aᵢapp} \quad (3) \]

Leaf life span and structural analyses

Leaf life span as used in this study refers to the average leaf life span (Lᵢ, years). Previously published values of Lᵢ were available for 15 out of the 35 species, all estimated at Kuring-gai Chase from repeat-census data collected over ≥2 years (Wright and Westoby, 2002; Read et al., 2006). While precise estimation of Lᵢ requires determination of leaf survivorship functions (e.g. Wright and Westoby, 2002; Reich et al., 2004), this information is rarely available, and Lᵢ can be estimated as the oldest leaf age class with at least 50% of leaves remaining, across many branches/plants (Cordell et al., 2001; Kayama et al., 2002) (Prior et al., 2003; Veneklaas and Poot, 2003 for Australian species). It was possible to estimate Lᵢ for 18 of the 20 remaining species using a combination of this cohort approach and knowledge of the species’ phenology. Only in the two cycad species, Macrozamia communis and M. spiralis, distinct leaf cohorts could not be reliably separated and thus leaf life span could not be determined.

All leaves used for the gas-exchange analyses and additional 4–20 representative leaves per twig (on average 6.3 leaves per twig) were taken for structural and chemical analyses. All leaves were scanned at a resolution of 300 dpi, and leaf area was estimated by UTHSCSA Imagentool 2.00alpha (C Donald Wilcox, S Brent Dove, W Doss McDavid and David B Greer, Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio, TX, USA; ddsdx.uthscsa.edu). For the gas-exchange leaves and for 4–12 additional leaves (on average 5.9 leaves per twig), leaf thickness (T) was measured with precision calipers at 2–7 (on average 4.8) separate leaf locations, and leaf-specific averages, averages for gas-exchange leaves, and whole-twig averages were calculated. The fresh mass of each leaf was thereafter determined to the nearest 0.1 mg, the leaves were further oven-dried at 65 °C for no less than 48 h, and the dry mass of each leaf was determined. The dry to fresh mass ratio (Dᵢ), dry mass per unit area (Mᵢ), and leaf density (s=Mᵢ/T) were obtained for each leaf, and separate averages for gas-exchange leaves and all leaves per twig were calculated.

Chemical analyses

Foliage total carbon and nitrogen contents were determined in fine-ground samples using a LECO CNS2000 Analyzer (LECO Corporation, St Joseph, MI, USA), while the P content was determined according to Raymond and Higgins (1992) after digestion of samples in a mixture of HNO₃ and HCl (1:1) by inductively coupled plasma emission spectroscopy (ICP-OES) using American Public Health Association standard method 3120 (APHA 3120). The same methods were used for chemical analysis of soil samples. Fractionation of leaf material into separate fibrous ‘fluff’ and powder components occurred during grinding in Banksia integrifolia, B. marginata, B. oblongifolia, and B. spinulosa. For these four species, the masses of fluff and powder were determined after grinding, and C, N, and P contents were estimated separately for these components. Whole-leaf average elemental composition was found as the mass-weighted average of leaf fluff and powder.

Data analyses

As area-based traits are the products of mass-based traits and Mᵢ, the correlations between both the area- and mass-based photosynthetic potentials (e.g. Vₑ(max)/area and Vₑ(max)/mass) were analysed as is common in leaf structure/function studies. However, the diffusion conductances are generally only expressed per unit area. This is justified for stomatal conductance, as gaseous transport between ambient atmosphere and the leaf surface occurs through stomatal pores on the leaf surface. However, mesophyll diffusion conductance is inherently a three-dimensional process (Parkhurst, 1994), and should therefore more effectively scale with the mesophyll exposed surface area (Nobel, 1991). Thus, CO₂ drawdown from substomatal cavities to chloroplasts is the leaf volume-weighted average not the leaf surface-weighted average (Ninemets and Sack, 2006; Ninemets et al., 2009). Thus, the scaling of mesophyll diffusion conductance per unit foliage mass with leaf structural traits and photosynthetic potentials per mass was also analysed.

A conservative statistical strategy was used with species-specific average trait values as independent observations. The main emphasis in the current study was on testing for structural and physiological controls on diffusional limitations of photosynthesis for a wide range of foliage
structures and physiological potentials. For this, linear and non-linear asymptotic [$y=a+bx+Log(x)$ and $y=ax^b$] regression analyses were conducted using all species pooled. Data from different sites were generally overlapping, and co-variation analyses with linearized relationships did not reveal site main effects. Nevertheless, for visual inspection of the data, different symbols are used in the figures to denote the sites. As the climatic and soil conditions of Macquarie University campus forest were similar to those of the wetter high nutrient site, and also the foliage traits were similar between these two sites, the same symbols were used for these sites in bivariate correlations.

Site differences were tested for using ANOVA. As just three species were available from the low rain/low nutrient site, ANOVA was conducted only for the three other sites. The average coefficient of variation of structural, chemical, and physiological characteristics was calculated as a measure of within-site variability. These average site-specific coefficients of variation were compared by paired t-tests. All statistical effects are considered significant at $P < 0.05$.

**Results**

**Basic characteristics of the ‘leaf economics spectrum’ for 35 Australian species**

Species mean leaf life span ($L_A$) varied 8.5-fold across all species (0.52–4.4 years) and leaf dry mass per unit area ($M_A$) varied 4.7-fold (66–313 g m$^{-2}$) (Appendix I). The variation in $M_A$ was attributed to the variation of both of its components, leaf density ($s$, ~2-fold variation, 0.29–0.56 g cm$^{-3}$) and thickness ($T$, ~2-fold variation, 274–594 µm). Nitrogen ($N_M$, 0.31–2.38%) and phosphorus ($P_M$, 0.0109–0.0869%) contents per dry mass varied almost 8-fold (Appendix I). Foliage biochemical potentials, the maximum Rubisco carboxylase activity ($V_{cmax}$, 0.076–0.729 µmol g$^{-1}$ s$^{-1}$), and the capacity for photosynthetic electron transport ($J_{max}$, 0.204–1.66 µmol g$^{-1}$ s$^{-1}$) on a dry mass basis varied 8- to 9-fold, while the area-based characteristics varied 4- to 5-fold across the species (Appendix I). The relationships among foliage longevity, and structural, chemical, and physiological traits reflected the worldwide ‘leaf economics spectrum’ (Wright et al., 2004b), i.e. leaves with greater longevity (slow-return end of the spectrum) had higher $M_A$, lower nutrient contents, and lower photosynthetic potentials, while short-living leaves (fast-return end of the spectrum) had less robust structure and had higher nutrient contents and photosynthetic capacities (Appendix II and Fig. 1). The correlations of foliage chemistry and photosynthetic potentials with foliage structural traits were stronger without nitrogen-fixing species (Leguminosae and Zamiaceae) that had greater $M_A$ and lower photosynthetic potentials at a given $N_M$.

Overall, the relationships were stronger with traits expressed per unit leaf dry mass (Appendix II and Fig. 1a, b), indicating that the negative effects of $M_A$ on mass-based nutrient contents and photosynthetic characteristics were quantitatively more important than the accumulation of photosynthetic biomass with increasing $M_A$ (any area-based trait is the product of mass-based trait and $M_A$).

**Mesophyll diffusion conductance versus foliage physiological and structural traits**

Across the species, mesophyll diffusion conductance ($g_{m}$) per unit area ($g_{m}$/area) varied 6-fold, and mass-based $g_{m}$

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**Fig. 1.** Correlations of mass- (a) and area-based (b) photosynthetic capacities with nitrogen content in 35 Australian species (see Appendix I for species and Table 1 for sites). Foliage photosynthetic capacity was simulated using the fully parameterized photosynthesis model of Farquhar et al. (1980) ($V_{cmax}$, $V_{mmax}$, dark respiration rate) for incident quantum flux density of 1000 µmol m$^{-2}$ s$^{-1}$, leaf temperature of 25 °C, and setting either the CO$_2$ concentration in substomatal cavities at 250 µmol mol$^{-1}$ (open symbols, $A_{dl}$) or the CO$_2$ concentration in chloroplasts at 250 µmol mol$^{-1}$ (filled symbols, $A_{app}$). $A_{dl}$ provides a standardized estimate of photosynthetic capacity that is independent of interspecific and site-specific variation in stomatal conductance, while $A_{app}$ provides a hypothetical estimate for infinite mesophyll diffusion conductance ($C_l=C_C$). Every data point corresponds to each individual species. The ellipse in (a) and rectangle in (b) enclose the data for the six N-fixing species that were outliers in bivariate correlations (Appendix II). Data were fitted separately with N-fixing species included (solid lines) and without these species (dashed lines) by non-linear regressions in the form of $y=ax^b$ (all are significant at $P < 0.01$). Analogous relationships, albeit somewhat weaker, were observed for non-standardized assimilation rates (data not shown).
(gm/mass) 12-fold. gm/area scaled positively with Vcmax/area (Fig. 2a) and Jmax/area (Fig. 2c), and gm/mass with Vcmax/mass (Fig. 2b) and Jmax/mass (Fig. 2d), but these correlations were stronger for mass-based relationships. Area-based gm and stomatal conductance were positively but weakly correlated (r²=0.23, P<0.005).

Mass-based gm was negatively associated with MA (r²=0.53; Fig. 3a), density (r²=0.34, P<0.001), thickness (r²=0.26, P<0.001), and leaf life span (Fig. 3b). gm/area relationships with leaf life span and structure were much weaker. Although weakly, gm/area was negatively correlated with Ll (r²=0.22, P<0.005), but not with MA, density, and thickness (P>0.1).

CO₂ concentration ratios, drawdowns, and reduction of photosynthesis due to finite gm

The ratio of CO₂ concentrations in chloroplasts to substomatal cavities, CC/Ci, varied 1.5-fold among species, while the CO₂ drawdown from substomatal cavities to

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**Fig. 2.** Internal diffusion conductance to CO₂ (gm, Eq. 2) per unit area (a, c) and dry mass (b, d) in relation to area- and mass-based maximum Rubisco carboxylase activity, Vcmax, (a, b) and the capacity for photosynthetic electron transport, Jmax, (c, d) in 35 Australian species. The area-based characteristics are the products of mass-based characteristics and leaf dry mass per unit area (MA). Each data point corresponds to the average of a given species, and error bars show ±SE. Data were fitted by linear and non-linear regressions in the form of y=ax² and y=a+bLog(x) whichever provided the stronger fit (larger r²). The description of the study sites is provided in Table 1. In the figures, data for six species from Macquarie University campus (high rain/rich soils) are pooled with those from the Ku-ring-gai Chase National Park high rain/high nutrient site (Table 1). Data presentation and fitting are as in Fig. 1.

**Fig. 3.** Mesophyll diffusion conductance per dry mass dependent on leaf dry mass per unit area (MA) (a) and average foliage life span (b) in 35 Australian species. Data presentation follows Fig. 2. Data were fitted by non-linear regressions in the form of y=ax².
sub stomatal cavities to chloroplasts, \( C_{\text{i}} - C_C \) varied 2.7-fold. \( C_C / C_i \) was negatively associated with \( M_A \) (\( r^2 = 0.32 \), Fig. 4a), leaf density (\( r^2 = 0.29 \)), dry to fresh mass ratio (\( r^2 = 0.28 \)), and leaf life span (\( r^2 = 0.40 \), \( P < 0.001 \) for all), indicating greater diffusional limitations in structurally more robust leaves. CO2 drawdown, \( C_C - C_i \), that scales positively with the diffusional limitations was positively linked to all these characteristics, albeit that the correlations were somewhat weaker (e.g. Fig. 4b for \( M_A \)). Neither \( C_C / C_i \) (\( r^2 = 0.05 \), \( P < 0.1 \)) nor \( C_C - C_i \) (\( r^2 = 0.03 \), \( P > 0.3 \)) was correlated with leaf thickness.

\( C_C / C_i \) was positively (Fig. 4c) and \( C_C - C_i \) negatively (Fig. 4d) correlated with \( g_m / \text{mass} \), indicating a lower degree of limitation of photosynthesis by internal diffusion in leaves with greater diffusion conductance. The correlations were analogous with \( g_m / \text{area} \), but weaker (\( r^2 = 0.15 \), \( P = 0.02 \) for \( C_C / C_i \) and \( r^2 = 0.21 \), \( P < 0.01 \) for \( C_C - C_i \)). The correlation of \( C_C / C_i \) and \( C_C - C_i \) with net assimilation rate and foliage physiological potentials, \( V_{\text{max}} \) and \( J_{\text{max}} \), was poor, with a significant correlation observed only for \( J_{\text{max}} / \text{mass} \) (\( r^2 = 0.20 \), \( P < 0.01 \) for both the negative correlation with \( C_C / C_i \) and the positive correlation with \( C_C / C_i \)).

The substomatal to ambient CO2 concentration ratio, \( C_C / C_i \), that characterizes the degree of stomatal limitation of photosynthesis, and \( C_C / C_i \) were not correlated (\( r^2 = 0.00 \), \( P > 0.7 \)). \( C_C / C_i \) was also not correlated with \( g_m \) on either an area or a mass basis (\( r^2 < 0.03 \), \( P > 0.2 \)). Nevertheless, the variability in \( C_C / C_i \) from \( \sim 0.5 \) to 0.8 was relatively large across the species, reflecting differences in stomatal openness during the measurements. When this variability among species in stomatal openness was taken into account by setting \( C_i \) to an average value of 250 \( \mu \text{mol} \text{ mol}^{-1} \), the correlations of \( C_C / C_i \) and \( C_C - C_i \) with foliage structural traits and \( g_m \) were in most cases improved (insets in Fig. 4).

Despite the interspecific variability in \( C_i \), the overall diffusion limitation characterized by the CO2 drawdown from the ambient air to chloroplasts, \( C_a - C_C \), was still lower in leaves with greater \( g_m / \text{mass} \) (\( r^2 = 0.20 \), \( P < 0.01 \)) and larger in leaves with greater \( M_A \) (\( r^2 = 0.21 \), \( P < 0.01 \)). Determination of a hypothetical foliage photosynthetic capacity for infinite \( g_m \) demonstrated that \( g_m \) did significantly alter realized foliage photosynthesis rates (Fig. 1). Relative reduction of photosynthesis at current ambient CO2 concentration due to finite diffusion conductance, \( A_{\text{DF}} \) (Eq. 3), was larger in structurally more robust leaves (Fig. 5a for correlation with \( M_A \)) and in leaves with lower mesophyll diffusion conductance (Fig. 5b).

**Site effects**

Due to limited species sampling at the low rain/low nutrient site, statistical comparisons were restricted to three sites. Among these sites, leaf life span was larger in the high rain/low nutrient site than in the other sites (Table 2). \( M_A \) was

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**Fig. 4.** Chloroplastic (\( C_C \)) to internal (sub stomatal cavities, \( C_i \)) CO2 concentration ratio, \( C_C / C_i \) (a, c) and CO2 drawdown from sub stomatal cavities to chloroplasts, \( C_i - C_C \) (b, d) in relation to \( M_A \) (a, b) and \( g_m / \text{mass} \) (c, d) in 35 Australian species. The values of \( C_C / C_i \) and \( C_C - C_i \) in the main panels are calculated using the actual \( C_i \) and \( C_C \) obtained for the ambient CO2 concentration range of 320–420 \( \mu \text{mol} \text{ mol}^{-1} \). Insets demonstrate the correlations obtained when \( C_i \) was fixed at 250 \( \mu \text{mol} \text{ mol}^{-1} \) for all leaves, and the net assimilation rate (\( A_{\text{DF}} \)) was calculated according to the photosynthesis model of Farquhar et al. (1980). Data presentation and fitting are as in Fig. 2.
also larger in this site, mainly because of greater leaf thickness (Table 2). Foliage nitrogen contents were statistically not different among the sites, but \( P_M \) was lower in the high rain/low nutrient site (Table 2), agreeing with the low soil P availability in this site (Table 1). The average coefficient of variation (standard deviation per mean of the given trait, in percent) of these key structural and chemical traits was similar across the sites, being lower only for the low rain/low nutrient site where fewer species were studied (Table 2). Thus, within most sites, a similar amount of variation among the species was discovered.

Area-based photosynthetic potentials and net assimilation rates were not statistically different among the sites, but \( V_{\text{max/mass}}, J_{\text{max/mass}}, \) and \( A_{\text{max/mass}} \) were lower in the high rain/low nutrient site (Table 2), agreeing with the low soil P availability in this site (Table 1). The average coefficient of variation (standard deviation per mean of the given trait, in percent) of these key structural and chemical traits was similar across the sites, being lower only for the low rain/low nutrient site where fewer species were studied (Table 2). Thus, within most sites, a similar amount of variation among the species was discovered.

Mesophyll diffusion conductance per area was smaller in the low rain/high nutrient site than in the high rain/high nutrient site, while \( g_m/\text{mass} \) was lower in both the high rain/low nutrient and low rain/high nutrient sites than in the high rain/high nutrient site (Table 3b). These differences in \( g_m/\text{mass} \) were accompanied by a lower \( C_C/C_i \) ratio and greater CO₂ drawdown in the high rain/low nutrient and low rain/high nutrient sites than in the high rain/high nutrient site (Table 3b). The coefficient of variation of the 11 traits in Table 3 (CV_p) was of similar magnitude to that for the structural and chemical traits in Table 2. CV_p tended to be lower in low nutrient sites (Table 3).

**Discussion**

**Foliage structure, chemistry, photosynthesis, and longevity at the lower end of the leaf economics spectrum**

Extremely low values of foliage N content per mass of 0.31% and P content per mass of 0.0109% were observed in actively photosynthesizing fully mature leaves. This, in combination with high values of foliage dry mass per unit area (\( M_A \)) of up to 313 g m⁻² and a high average life span, means that the studied species are positioned in the low nutrient/high \( M_A \) end of the leaf economics spectrum (Appendix II, Wright et al., 2004b). Low foliage nutrient contents per mass reflect extreme soil nutrient deficiencies in
Australian old highly leached soils (Specht, 1969; di Castri, 1981). Similar temperate shrublands supporting evergreen broad-leaved vegetation are found in more fertile soils in the Americas and Europe (di Castri, 1981). Although species were sampled from ‘high’ and ‘low’ nutrient sites, compared with other temperate broad-leaved world ecosystems, the overall soil nutrient availability was low in all ecosystems studied.

Low values of nutrient contents per mass and high $M_A$ resulted in low foliage photosynthetic potentials per unit dry mass, agreeing with worldwide patterns (Appendix II and Fig. 1a; Wright et al., 2004b). Despite low mass-based values, photosynthetic capacities as high as 10 μmol m$^{-2}$ s$^{-1}$ were achieved as the result of accumulation of N and mesophyll per unit leaf area in thick leaves (Fig. 1b). These values are comparable with photosynthetic activities of broad-leaved evergreens from more nutrient-rich sites in the Americas and Europe, but significantly less than those in broad-leaved temperate deciduous species (Ellsworth et al., 2004).

Adaptation of Australian species to nutrient deficiencies has been linked to specific features such as cluster roots and extremely high nutrient use efficiency in Proteaceae (Lamont, 1993; Denton et al., 2007). Foliar sclerophyll of Australian species as manifested in high $M_A$ values has also been interpreted as mainly indicative of nutrient limitations (Loveless, 1961; Specht and Rundel, 1990). Specifically, leaves with higher $M_A$ are tougher (Read andanson, 2003; Read et al., 2005) and more resistant to mechanical damage and herbivory. Thus, they possess higher longevity and greater nutrient retention time. In several widespread angiosperm families in Australia such as Proteaceae and Casuarinaceae, leaf mesophyll is often present as mesophyll ‘islands’ embedded in a highly lignified mesophyll structure (Blackman et al., 2005; Jordan et al., 2005; Niinemets et al., 2005b), supporting the hypothesis that high $M_A$ leaves have more developed mechanical defences.

Limited water availability (Hill, 1998; Lamont et al., 2002; Mast and Givnish, 2002) as in comparable evergreen broad-leaved ecosystems in the Americas and Europe may also partly explain the sclerophyllous leaf habit in Australian species. Recently it has been suggested that the scleromorphic leaf structure of Australian species, characterized by several epidermal/hypodermal leaf layers on the upper leaf surface and overall high leaf thickness, may reflect adaptation to high solar irradiances that occur in nutrient-limited open shrublands (Smith et al., 1998; Jordan et al., 2005). In fact, a large variability in foliage anatomy exists among Australian sclerophylls (Blackman et al., 2005; Jordan et al., 2005) that is also reflected in integral characteristics such as the components of $M_A$, leaf thickness, and density ($M_A$=density×thickness). Within species (Groom and Lamont, 1997) and among species (Niinemets, 2001), density tends to increase with decreasing water availability, while thickness scales positively with irradiance, but this relationship obviously may depend on specific anatomical modifications that alter the distribution of foliage biomass between mesophyll and the support structure and the density of each leaf fraction (Poorter et al., 2009). In the present study, $M_A$ was correlated with both thickness and density that both varied ~2.5-fold (Appendix II), indicating that

<table>
<thead>
<tr>
<th>Table 3. Foliage photosynthetic potentials (a) and mesophyll diffusion conductance to CO$_2$ and reductions in CO$_2$ concentration due to diffusion in species from four contrasting environments$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Maximum carboxylase activity of Rubisco ($V_{\text{cmax}}$, capacity for photosynthetic electron transport ($J_{\text{max}}$), and light-saturated net assimilation rate ($A_{\text{max}}$) on area (μmol m$^{-2}$ s$^{-1}$) and mass (μmol g$^{-1}$ s$^{-1}$) basis</td>
</tr>
<tr>
<td>Resource availability</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>High</td>
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<tr>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
</tr>
</tbody>
</table>

(b) Mesophyll diffusion conductance to CO$_2$ ($g_m$) per area ($g_m$/area. μmol m$^{-2}$ s$^{-1}$), and per mass ($g_m$/mass, μmol g$^{-1}$ s$^{-1}$), and the ratios of CO$_2$ concentrations in substomatal cavities to ambient air ($C_i/C_a$), chloroplasts ($C_i/C$) to that in substomatal cavities ($C_i/C$), CO$_2$ drawdown $C_i-C_a$ (μmol μmol$^{-1}$), and average coefficients of variation (CV) of the 11 traits in (a) and (b)

| Resource availability | Variable | Water | Nutrients | $g_m$/area | $g_m$/mass | $C_i/C_a$ | $C_i/C$ | $C_i-C_a$ | CV$^*$ |
| High | High | 0.087±0.010 μmol m$^{-2}$ s$^{-1}$ | 0.74±0.15 μmol g$^{-1}$ s$^{-1}$ | 0.637±0.028 μmol g$^{-1}$ s$^{-1}$ | 0.692±0.018 μmol g$^{-1}$ s$^{-1}$ | 77±5 μmol m$^{-2}$ s$^{-1}$ | 36±6 μmol g$^{-1}$ s$^{-1}$ |
| High | Low | 0.071±0.006 μmol m$^{-2}$ s$^{-1}$ | 0.396±0.041 μmol g$^{-1}$ s$^{-1}$ | 0.695±0.019 μmol g$^{-1}$ s$^{-1}$ | 0.630±0.014 μmol g$^{-1}$ s$^{-1}$ | 93±6 μmol m$^{-2}$ s$^{-1}$ | 23.4±2.9 μmol g$^{-1}$ s$^{-1}$ |
| Low | High | 0.052±0.008 μmol m$^{-2}$ s$^{-1}$ | 0.31±0.08 μmol g$^{-1}$ s$^{-1}$ | 0.654±0.034 μmol g$^{-1}$ s$^{-1}$ | 0.616±0.024 μmol g$^{-1}$ s$^{-1}$ | 96±5 μmol m$^{-2}$ s$^{-1}$ | 30.9±4.5 μmol g$^{-1}$ s$^{-1}$ |
| Low | Low | 0.065±0.011 μmol m$^{-2}$ s$^{-1}$ | 0.36±0.06 μmol g$^{-1}$ s$^{-1}$ | 0.520±0.022 μmol g$^{-1}$ s$^{-1}$ | 0.70±0.05 μmol g$^{-1}$ s$^{-1}$ | 76±5 μmol m$^{-2}$ s$^{-1}$ | 25.4±2.9 μmol g$^{-1}$ s$^{-1}$ |

* Data presentation and statistical analysis are as in Table 2.
*$^*$ Standardized to $C_i=250$ μmol μmol$^{-1}$. 


different selection forces may have operated on foliage structure.

**Mesophyll diffusion conductance (g_m) in relation to structural and physiological traits**

In addition to nutrient contents and nutrient use efficiency that determine the biochemical foliage photosynthetic potentials, the realized net assimilation rates of leaves with given biochemical capacities and given openness of stomatal pores also depend on the reduction of CO\textsubscript{2} from substomatal cavities to chloroplasts. It was observed that g\textsubscript{m} scaled positively with foliage photosynthetic potentials on both an area and a mass basis (Fig. 2). Such positive relationships have been shown in several studies (Evans and Loreto, 2000; Niinemets and Sack, 2006; Flexas et al., 2008; Warren, 2008), and reflect a greater number of chloroplasts and higher chloroplast surface area for diffusion in leaves with a greater concentration of photosynthetic machinery and a larger number of mesophyll cell layers (Evans et al., 1994; Syvertsen et al., 1995; Terashima et al., 2006). While previous studies have mostly considered the correlations between area-based photosynthetic potentials and g\textsubscript{m}, in the present study the correlations for a structurally and chemically widely varying set of species were actually stronger on a mass basis (Appendix II, compare Fig. 1a, b and Fig. 2a, b). Given that CO\textsubscript{2} drawdown from the substomatal cavities to chloroplasts is a mesophyll volume-weighted average (Niinemets and Sack, 2006), a stronger correlation between mass-based a mesophyll volume-weighted average (Niinemets and Sack, 2006), demonstrating that the reduction of g\textsubscript{m} at the higher end of M\textsubscript{A} is asymptotic.

The average g\textsubscript{m}/area values of 0.052–0.087 mol m\textsuperscript{-2} s\textsuperscript{-1} observed in the present study (Table 3) are similar to those in other broad-leaved evergreen sclerophyll species (Niinemets et al., 2005a; Flexas et al., 2008). However, average M\textsubscript{A} values in this study are somewhat larger than in the other studies with sclerophylls (compare Table 2 and Niinemets et al., 2005a; Flexas et al., 2008). Greater g\textsubscript{m} values at a given M\textsubscript{A} in this study may reflect the circumstance that sclerophylly in European species has mainly evolved in response to drought, while several other factors including nutrient conservation have played a major role in sclerophylly in Australian species (see above). While thick-walled mesophyll is distributed uniformly between the epidermal layers in European sclerophylls (Christodoulakis and Mitrakos, 1987), heterogeneous distribution of thick-walled sclerenchyma and mesophyll islands, where individual cell walls do not necessarily have thick walls, is characteristic of Australian sclerophylls (Jordan et al., 2005). The contributions of thickness and density to M\textsubscript{A} do differ among Australian species (Witkowski and Lamont, 1991). In addition, leaves of a given M\textsubscript{A} may vary widely in the way mesophyll and support structures are arranged in the leaves and in the average cell wall thickness of mesophyll, epidermal, hypodermal, and sclerenchyma cells.

**Diffusional limitations of foliage photosynthesis in Australian sclerophylls**

Given the positive correlations between photosynthetic potentials and g\textsubscript{m} on both a mass and an area basis, and the negative correlation of both g\textsubscript{m}/mass and photosynthetic potentials per mass with M\textsubscript{A}, the crucial question is to what extent the negative relationship between g\textsubscript{m} and M\textsubscript{A} results in differences in CO\textsubscript{2} drawdown from the substomatal cavities to chloroplasts (C\textsubscript{i}–C\textsubscript{c}). It has been suggested previously that the positive correlation, often linear, between photosynthetic capacity and g\textsubscript{m} implies that the ratio of the realized net assimilation rate to g\textsubscript{m} (A/g\textsubscript{m}), i.e. CO\textsubscript{2} drawdown (A/g\textsubscript{m}=C\textsubscript{i}–C\textsubscript{c}), is relatively invariant across the species of differing photosynthetic capacities and leaf structures (Evans and Loreto, 2000). Recent studies, however, have highlighted that C\textsubscript{i}–C\textsubscript{c} scales negatively with g\textsubscript{m} and is larger in structurally more robust leaves (Niinemets et al., 2005a; Niinemets and Sack, 2006; Warren and Adams, 2006). In the present study, the variation in the variables characterizing diffusional...
limitations of photosynthesis, the $C_C/C_i$ ratio and $C_i-C_C$, was 1.5- to 2.7-fold across the studied species (Fig. 4). Although this is less than in foliage structural and physiological traits (Appendix II), the diffusional limitations were larger in leaves with lower $g_m$/mass (Fig. 4c, d). The diffusional limitations were also larger in leaves with greater $M_A$ and longevity (Fig. 4a, b). Finding such broad patterns within a structurally highly diverse data set conclusively demonstrates that limited diffusion conductance does curb photosynthesis more strongly in leaves with more robust structure whether the measure of leaf robustness is $M_A$ or leaf density.

Average CO$_2$ drawdowns from $C_i$ to $C_C$ observed in this study, 50–125 μmol mol$^{-1}$, are somewhat lower than for European Mediterranean sclerophylls with comparable structure but larger foliar $N_M$ (Niinemets et al., 2005a, 2006; Warren, 2008). Nevertheless, the relative reduction of photosynthesis due to finite diffusion conductance at current ambient CO$_2$ concentration, $A_D$ (Eq. 3), was large, being $>0.5$ in most sclerophyllous leaves, but in no cases <0.2 (Figs. 1, 5). Obviously, this loss of potential assimilation is the cost these leaves pay for tolerating extreme nutrient deficiencies and water limitations.

### Ecosystem-level differences in foliage function and diffusional limitations

Previous studies have highlighted important shifts in foliage functioning across nutrient and rainfall contrasts in Australian species. In particular, species from low rain sites have been shown to have lower realized photosynthesis at a given foliage N and P, and thus were suggested to operate at greater CO$_2$ drawdowns from ambient air to the substomatal cavities, partly as the result of differences in stomatal openness between high rain versus low rain sites (Wright et al., 2001). In the present study, species from different sites were generally overlapping in all bivariate relationships (Appendix II, Figs. 2–5), but it is also important to consider that the precipitation contrast, an ~1.5-fold difference in this study (Table 1), was smaller than the contrast of ~3-fold in previous studies (Wright et al., 2001).

In addition to scaling relationships, it is also relevant to understand whether the collections of species in any given site differ in average mesophyll diffusion limitations of photosynthesis. The main contrasts in this study were a lower average $M_A$ and leaf life span (Table 2) and higher photosynthetic potentials per dry mass (Table 3a) in the high rain/high nutrient site. Higher mass-based assimilation capacities were mainly associated with lower $M_A$ as area-based capacities did not differ among sites (Table 3). Lower site-average $M_A$ and greater mass-based photosynthetic potentials were also associated with higher $g_m$/mass and lower CO$_2$ drawdowns from substomatal cavities to chloroplasts in this site (Table 3). To our knowledge, this is the first report demonstrating site differences in average mesophyll diffusion limitations of photosynthesis. This suggests that sites supporting species with different foliage architectures may have different photosynthetic sensitivities to variation in ambient CO$_2$ concentrations.

### Conclusions

This study provides information of foliage photosynthetic potentials and limitations of net assimilation rate by mesophyll diffusion conductance for 35 species from the low nutrients/high $M_A$ and life span end of the global spectrum of leaf functioning. The study demonstrated large variation in the mesophyll diffusion limits of photosynthesis across the species of contrasting structure, chemistry, and photosynthetic capacity, with CO$_2$ mesophyll diffusion limitations being larger in species with more robust foliage structure. As CO$_2$ drawdown from substomatal cavities to chloroplasts is a volume-weighted average, CO$_2$ diffusion limitations scaled more strongly with mass-based mesophyll diffusion conductance. Having a robust structure with the advantages of being more tolerant to a variety of abiotic and biotic stresses and living longer has the inevitable disadvantage of being less efficient in mesophyll diffusion. In addition to species differences, sites supporting species with more robust structure such as low rain/high nutrient and low rain/high nutrient sites versus the high rain/high nutrient site in this study, photosynthesis is more strongly limited by CO$_2$ diffusion in the mesophyll.

The values of mesophyll diffusion conductance were somewhat larger and those of CO$_2$ drawdowns lower than observed for broad-leaved evergreen species from comparable ecosystems in other continents. This difference probably reflects the evolution of sclerophyllous structure in Australian species primarily in response to limited soil nutrient availability, while in other ecosystems this occurred primarily in response to limited water availability. As a result, many Australian species possess unique leaf architectures, where ‘mesophytic’ mesophyll islands not necessarily exhibiting thick cell walls are embedded in highly lignified support tissue. Such unique leaf architectural variations need consideration when general relationships are sought among foliage diffusion conductance, CO$_2$ drawdown, and bulk leaf structural characteristics such as leaf dry mass per unit area (Poorter et al., 2009).

### Acknowledgements

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Appendix I. List of studied species with key foliage traits

<table>
<thead>
<tr>
<th>Species*</th>
<th>Site†</th>
<th>Life span (years)</th>
<th>Dry mass per area (g m⁻²)</th>
<th>Thickness (mm)</th>
<th>Density (g cm⁻³)</th>
<th>N content (%)</th>
<th>P content (%)</th>
<th>Photosynthetic capacity (μmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia falcata</td>
<td>LRHN</td>
<td>2.0</td>
<td>131.6</td>
<td>0.270</td>
<td>0.486</td>
<td>1.93</td>
<td>0.0531</td>
<td>4.45</td>
</tr>
<tr>
<td>Acacia longifolia</td>
<td>MQ</td>
<td>2.0</td>
<td>117.9</td>
<td>0.331</td>
<td>0.357</td>
<td>2.38</td>
<td>0.0411</td>
<td>4.45</td>
</tr>
<tr>
<td>Acacia myrtifolia</td>
<td>HRLN</td>
<td>1.5</td>
<td>145.2</td>
<td>0.357</td>
<td>0.407</td>
<td>1.66</td>
<td>0.0148</td>
<td>7.59</td>
</tr>
<tr>
<td>Acacia suaveolens</td>
<td>HRLN</td>
<td>2.5</td>
<td>170.9</td>
<td>0.447</td>
<td>0.382</td>
<td>2.11</td>
<td>0.0289</td>
<td>4.93</td>
</tr>
<tr>
<td>Angophora hispida</td>
<td>HRLN</td>
<td>2.5</td>
<td>178.9</td>
<td>0.386</td>
<td>0.461</td>
<td>0.60</td>
<td>0.0367</td>
<td>4.50</td>
</tr>
<tr>
<td>Astronicha floribunda</td>
<td>HRLN</td>
<td>0.5</td>
<td>66.1</td>
<td>0.237</td>
<td>0.281</td>
<td>1.84</td>
<td>0.0626</td>
<td>9.19</td>
</tr>
<tr>
<td>Banksia aemula</td>
<td>LRLN</td>
<td>3.0</td>
<td>178.1</td>
<td>0.405</td>
<td>0.445</td>
<td>0.50</td>
<td>0.0208</td>
<td>4.50</td>
</tr>
<tr>
<td>Banksia integrifolia</td>
<td>MQ</td>
<td>2.2</td>
<td>229.8</td>
<td>0.541</td>
<td>0.439</td>
<td>0.72</td>
<td>0.0412</td>
<td>8.33</td>
</tr>
<tr>
<td>Banksia marginata</td>
<td>HRLN</td>
<td>3.0</td>
<td>221.7</td>
<td>0.390</td>
<td>0.575</td>
<td>0.44</td>
<td>0.0109</td>
<td>5.04</td>
</tr>
<tr>
<td>Banksia oblongifolia</td>
<td>HRLN</td>
<td>2.6</td>
<td>258.3</td>
<td>0.428</td>
<td>0.605</td>
<td>0.75</td>
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<td>0.270</td>
<td>0.84</td>
<td>0.0624</td>
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<td>MQ</td>
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<td>172.6</td>
<td>0.380</td>
<td>0.453</td>
<td>0.62</td>
<td>0.0431</td>
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<td>0.504</td>
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<td>0.55</td>
<td>0.0250</td>
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<td>LRHN</td>
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<td>219.8</td>
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<td>4.39</td>
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<td>145.4</td>
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<td>211.6</td>
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<td>161.4</td>
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<td>0.0183</td>
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<td>0.547</td>
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<td>176.0</td>
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<td>144.5</td>
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<td>0.470</td>
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<td>0.0336</td>
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</table>

* Species nomenclature follows the Australian Plant Name Index (http://www.anbg.gov.au/databases/apni-about/).
† HRHN, wetter, high nutrients; HRLN, wetter, low nutrients; LRHN, drier, high nutrients; LRLN, drier, low nutrients (see Table 1 for site characteristics). MQ, Macquarie University campus, North Ryde, Sydney (33°46' S, 151°06' E).

Appendix II. Correlations between foliage longevity, structure, chemistry, and photosynthesis: ‘leaf economics spectrum’ for 35 Australian species

The traits associated with robust leaf structure, such as high leaf dry mass per unit area ($M_A$) and high life span ($L_L$), are commonly associated with low nutrient contents and low photosynthetic potentials, while leaves with low $M_A$ and $L_L$ typically have high nutrient contents and photosynthetic capacities (‘leaf economics spectrum’ Wright et al., 2004b), although important discrepancies from worldwide trends can occur within specific parts of the spectrum (Diemer, 1998; Wright et al., 2004a). In this data set characterizing the ‘slow-return’ end of the spectrum, leaf dry mass per unit area ($M_A$) was positively correlated with $L_L$ (Fig. A1a), and negatively with nitrogen ($N_M$, Fig. A1b) and phosphorus ($P_M$, $r^2=0.17$, $P <0.02$) contents per dry mass. The relationships with $N$ and $P$ were stronger without the six nitrogen-fixing species from Zormiaeae and Leguminosae ($r^2=0.47$, $P <0.001$ for $N_M$, $r^2=0.35$, $P <0.001$ for $P_M$) that tended to have larger $M_A$ at a given $N_M$ and $P_M$ (Fig. A1b). The correlation between $N_M$ and $P_M$ was positive, but weak ($r^2=0.24$, $P <0.005$).

The components of $M_A$, leaf density ($s$) and thickness, were not themselves correlated ($r^2=0.02$, $P >0.4$), but both of them contributed to the interspecific variation in $M_A$ (Fig. A1c, d). $T$ and $s$ were positively associated with $L_L$ ($r^2=0.20$, $P <0.01$ for $s$, and
For example, positively correlated with $P$, and $>0.1$ for $T$, but the negative scaling of $N_M$ (Fig. A1b) and $P_M$ with $M_A$ was attributed only to $s$ ($r^2=0.21$, $P<0.005$ for the negative correlation with $N_M$; $r^2=0.13$, $P<0.05$ for $P_M$). The correlations of $T$ with $N_M$ and $P_M$ were not significant ($P>0.1$). The foliage dry to fresh mass ratio ($D_f$) was positively correlated with $M_A$ ($r^2=0.39$) and $s$ ($r^2=0.81$, $P<0.001$ for both), but not with $T$ ($P>0.9$).

Area-based foliage N and P contents ($N_A$, $P_A$), the products of $N_M$, $P_M$, and $M_A$, were not significantly related to leaf structural traits (e.g. $r^2=0.01$, $P>0.5$ for $N_A$ versus $M_A$, and $r^2=0.05$, $P>0.1$ for $P_A$ versus $M_A$), indicating that the positive effect of biomass accumulation per unit area with increasing $M_A$ was of similar magnitude to the countervailing, negative effect of a reduced concentration of a given element in more robust leaves.

Foliage photosynthetic potentials, maximum carboxylase activity of Rubisco ($V_{cmax}$), and capacity for photosynthetic electron transport ($V_{jmax}$) per unit mass ($V_{cmax}/mass$ and $J_{max/mass}$) decreased with increasing $M_A$ (Fig. A2a, d) and $L_{A,b}$ ($r^2=0.52$ for $V_{cmax/mass}$ and $r^2=0.65$ for $J_{max/mass}$, $P<0.001$ for both), reflecting reductions in $N_M$ in leaves with a larger $M_A$ and longer life span (cf. Figs. A1b and A2b, e). The positive scaling of photosynthetic potentials with $N_M$ (Fig. A2b, e) was variable at higher $N_M$ values. This mainly reflected lower $V_{cmax}$ and $J_{max}$ values at a given N in six N-fixing species (Fig. A2b, e for regressions without N-fixing species).

$V_{cmax/mass}$ and $J_{max/mass}$ also scaled positively with $P_M$, but the relationships were weaker than with $N_M$ (cf. Fig. A2b, e and Fig. A2c, f). Separate fitting of $V_{cmax/mass}$ and $J_{max/mass}$ without N-fixing species also improved the correlations with $P_M$ ($r^2=0.29$ for $V_{cmax}$, and $r^2=0.35$ for $J_{max}$, $P<0.005$ for both). In all relationships, Astrotricha floccosa, the species with the shortest life span, had higher photosynthetic potentials at given $M_A$, $N_M$, and $P_M$ than the rest of the data (Fig. A2a).

Area-based $V_{cmax}$ and $J_{max}$, the products of mass-based variables and $M_A$, behaved similarly to mass-based quantities, but they were generally more weakly associated with foliage structural and chemical traits (Fig. A3 for $V_{cmax/mass}$/area; the explained variance in $J_{max}$ versus $M_A$, $N_A$, and $P_A$ was even somewhat lower), indicating that the negative effects of $M_A$ on mass-based chemical (Fig. A1) and physiological (Fig. A2) traits were quantitatively more important than the accumulation of photosynthetic biomass with increasing $M_A$. In contrast to the mass-based relations, the presence of N-fixing species did not alter the correlations of area-based photosynthetic potentials and $P_A$.

Although the trends of negative scaling of photosynthetic potentials and key nutrient contents with $M_A$ and leaf life span observed in the present study were in general agreement with broad worldwide patterns (Wright et al., 2004b), the photosynthetic potentials versus leaf structure and chemistry relationships were relatively scattered compared with worldwide trends in leaf functioning. Moderate degrees of explained variation and a certain lack of generality in these relationships in Australian species have also been observed in other studies (Wright et al., 2001; Wright and Westoby, 2002; Prior et al., 2003; Warren and Adams, 2004; Denton et al., 2007). Significant scatter partly reflects lower ranges of structural, chemical, and physiological variables in these nutrient- and water-limited sites. Foliage traits varied up to one order of magnitude in the current data set and in other Australian data sets (for an overview, see Wright et al.,
Fig. A2. Correlations of the maximum mass-based Rubisco carboxylase activity ($V_{\text{cmax}}$/mass) (a–c) and the capacity for photosynthetic electron transport ($J_{\text{max}}$/mass) (d–f) with leaf dry mass per area (a, d), nitrogen content per dry mass (b, e), and phosphorus content per dry mass (c, f) in 35 Australian species. Sites and symbols are as in Fig. A1. Data were fitted by linear and non-linear regressions in the form of $y=ax^b$ and $y=a+b\log(x)$ whichever provided the larger $r^2$. The ellipse in (b) and (e) denotes the six nitrogen-fixing species, and regressions without these species are shown by a dashed line. $A. f.$ denotes Astrotricha floccosa, the species with the shortest life span in the present data set (Appendix I) that had a larger photosynthetic capacity at a given foliage structure and nutrient content than the rest of the data.

Fig. A3. Relationships of maximum area-based Rubisco carboxylase activity ($V_{\text{cmax}}$/area) with $M_a$ (a), nitrogen (b), and phosphorus (c) contents per area in 35 Australian species. Data presentation and fitting are as in Fig. A2. Fits to the data in (b) are for all species pooled (solid line) and without N-fixing species (dashed line).

2004a) versus up to two orders of magnitude in the GLOPNET data set with worldwide species coverage (Wright et al. 2004b). In addition, part of the scatter was associated with N-fixing species from the Leguminosae and Zamiaceae (Figs. A1, A2). This is in agreement with previous studies that have demonstrated that certain species groups, such as Leguminosae or Myrtaceae, may stand out in terms of trait relationships in Australian species (Wright and Westoby, 1999; Prior et al., 2003; Wright and Westoby, 2003).

References


Li-Cor Inc. 2004. Using the LI-6400 portable photosynthesis system. Version 5. Lincoln, NE, USA: Li-Cor, Inc.


