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RESEARCH PAPER

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 (V_{cmax} and J_{max}), and mesophyll diffusion conductance (g_m) 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 g_m 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 (M_A) varied ~5-fold, leaf life span (L_L) and N (N_M) and P (P_M) 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 M_A and longevity were associated with lower nutrient contents and lower foliage photosynthetic potentials. g_m was positively correlated with V_{cmax} and J_{max}, and these correlations were stronger on a mass basis. Only g_m/mass was negatively associated with M_A. CO_2 drawdown from substomatal cavities to chloroplasts (C_i–C_C) characterizing mesophyll CO_2 diffusion limitations was larger in leaves with greater M_A, lower g_m/mass, and lower photosynthetic potentials. Relative limitation of A due to finite mesophyll diffusion conductance, i.e. 1–A(infinite g_m)/A(actual g_m), was always >0.2 and up to 0.5 in leaves with most robust leaf structure, demonstrating the profound effect of finite g_m 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 g_m 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 g_m, and demonstrate that realized photosynthesis rates are strongly limited by g_m 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; A_{app} (μmol m^{-2} s^{-1}), hypothetical A in the absence of g_m; A_{st} (μmol m^{-2} s^{-1}), A standardized to C_i = 250 μmol mol^{-1}; C_a (μmol mol^{-1}), ambient CO_2 concentration; C_C (μmol mol^{-1}), CO_2 concentration in chloroplasts; $C_{C,st}$ (μmol mol^{-1}), C_C standardized to C_i=250 μmol mol^{-1}; C_i (μmol mol^{-1}), CO_2 concentration in substomatal cavities; D_F (g g^{-1}), leaf dry to fresh mass ratio; g_m, mesophyll diffusion conductance to CO_2; g_m/area (mol m^{-2} s^{-1}), g_m per unit area; g_m/mass (mmol g^{-1} s^{-1}), g_m per unit dry mass; J_{ETR}, rate of photosynthetic electron transport from fluorescence (Eq. 1); J_{max}, capacity for photosynthetic electron transport; J_{max}/area (μmol m^{-2} s^{-1}), J_{max} per unit area; J_{max}/mass (μmol g^{-1} s^{-1}), J_{max} per unit dry mass; L_L (year), leaf life span; M_A (g m^{-2}), leaf dry mass per unit area; N_A (g m^{-2}), leaf nitrogen content per unit area; N_M (%), leaf nitrogen content per dry mass; P_A (g m^{-2}), leaf phosphorus content per area; P_M (%), leaf phosphorus content per dry mass; Q_{abs} (μmol m^{-2} s^{-1}), absorbed photosynthetic quantum flux density; R_d (μmol m^{-2} s^{-1}), non-photorespiratory respiration rate continuing in light; T (μm), leaf thickness; V_{cmax}, maximum carboxylase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco); V_{cmax}/area (μmol m^{-2} s^{-1}), V_{cmax} per unit area; V_{cmax}/mass (μmol g^{-1} s^{-1}), V_{cmax} per unit dry mass; $\mathbf{s}$, leaf density (M_A/T, g cm^{-3}); Γ^* (μmol mol^{-1}), hypothetical CO_2 compensation point of photosynthesis without R_d; Φ_{PSII} (mol mol^{-1}), effective quantum yield of photosystem II; Λ_D, relative limitation of photosynthesis due to g_m (Eq. 3).

Introduction

During photosynthesis, CO_2 diffusion from substomatal cavities to chloroplasts is limited by mesophyll diffusion conductance (g_m) that reduces the CO_2 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 ~20 mmol m^{-2} s^{-1} to 2000 mmol m^{-2} 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–C_C 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 CO_2 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 CO_2 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 CO_2 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 CO_2 drawdown (Niinemets *et al.*, 2005*a*, 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.*, 2005*a*; 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 ~120 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/mass than with the more traditionally used g_m/area (Niinemets *et al.*, 2005*a*; Niinemets and Sack, 2006), but g_m/mass versus g_m/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 ~180 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.*, 2004*a*; Denton *et al.*, 2007). It was suggested that these sclerophylls belong worldwide to the species groups with the largest CO_2 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 CO_2 drawdown, $C_i–C_C$ (A/g_m), 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 CO_2 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/mass than for g_m/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 CO_2 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

Table 1. Description of the study sites

Wetter sites were situated in Ku-ring-gai Chase National Park, and the drier sites in the Cumberland Plain (Agnes Banks Nature Reserve and Castlereagh Nature Reserve). Climatic data correspond to the nearest meteorological station and are >100 year averages according to the Bureau of Meteorology, Australian Government (http://www.bom.gov.au). Chemical data for Ku-ring-gai Chase National Park sites are from Wright *et al.* (2001).

Characteristic	Wetter sites		Drier sites	
	High nutrients	Low nutrients	High nutrients	Low nutrients
Location	33°34′S, 151°17′E	33°41′S, 151°08′E	33°39′S, 150°42′E	33°36′S, 150°43′E
Plant cover	Closed forest	Low open woodland	Open woodland	Low open woodland
Average overstorey height (m)	15–20	8–12	8–15	4–6
Overstorey dominants	*Eucalyptus umbra*, *Livistona australis*, *Syncarpia glomulifera*	*Angophora hispida*, *Corymbia gummifera*	*Angophora bakeri*, *Eucalyptus fibrosa*, *Eucalyptus umbria*	*Banksia aemula*, *Eucalyptus sclerophylla*
Annual rainfall/minimum rainfall of three driest months (mm)	1220/234	1220/234	801/133	801/133
Mean annual min/max temperature (°C)	22.5/13.3	22.5/13.3	23.9/10.5	23.9/10.5
Soil type	Red-brown clay	Yellow-grey sand	Sandy clay	Yellow sand
Soil parent rock	Weathered volcanic dyke	Hawkesbury sandstone	Tertiary alluvial deposits	Windblown dunes of Pliocene to Pleistocene
Total soil P (μg g^{-1}) ±SD	440±230	94±28	205±23	5±5
Total soil N (%) ±SD	0.26±0.15	0.030±0.001	0.059±0.007	<0.01

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 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 robur*, 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.* (2005*a*). 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.*, 2005*a*).

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-exchange/fluorescence measurements).

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 (CO_2 concentration in substomatal cavities, C_i) CO_2 response curves simultaneously with the effective quantum yields of photosystem II following the protocol of Niinemets *et al.* (2005*a*). 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 CO_2 of 50 μmol mol^{-1} during the leaf stabilization period was used to ensure maximum stomatal openness before the measurement of A/C_i curves (Centritto *et al.*, 2003). At this ambient CO_2 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 CO_2 concentrations between 50 μmol mol^{-1} and 2000 μmol mol^{-1}. When the steady-state assimilation rate at a given CO_2 concentration, ~5 min after change in CO_2 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 F_m'. From these measurements, the effective quantum yield of photosystem II, Φ_{PSII}, was calculated as $(F_m'-F)/F_m'$. Upon completion of the A/C_i curve, the light was switched off, and as soon as the chamber temperature and ambient CO_2 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 (J_{ETR}), 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, Q_{abs}. J_{ETR} was determined as (Schreiber *et al.*, 1994):

$$J_{ETR} = 0.5\Phi_{PSII}Q_{abs} \quad (1)$$

Determination of foliage gas-exchange characteristics

All foliage gas-exchange measurements were corrected for diffusion of CO_2 and water vapour through LI-6400 neoprene/polyethylene gaskets according to Rodeghiero *et al.* (2007). Mesophyll CO_2 diffusion conductance from the substomatal cavities to chloroplasts (g_m) was calculated as (Harley *et al.*, 1992):

$$g_m = \frac{A}{C_i - \frac{\Gamma^*[J_{ETR}+8(A+R_d)]}{J_{ETR}-4(A+R_d)}}, \quad (2)$$

where R_d is the non-photorespiratory respiration rate during the light period, and Γ* is the hypothetical CO_2 compensation point without R_d. Γ*=42.9 μmol mol^{-1} at 25 °C according to Bernacchi (2001). As previously (Niinemets *et al.*, 2005*a*, 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 g_m by curve fitting according to Ethier *et al.* (2004) gave estimates of g_m very similar to Eq. 2 (for a comparison between the two methods, see Niinemets *et al.*, 2005*a*).

Average values of g_m were computed for A values obtained for internal CO_2 concentrations of 150–350 μmol mol^{-1}. Over this C_i range, the values of g_m are stable, and the estimates of g_m are relatively insensitive to minor errors in Γ*, R_d, and A (Harley *et al.*, 1992; Niinemets *et al.*, 2006).

Chloroplastic CO_2 concentrations (C_C) for any given A were further calculated as $C_C=C_i-A/g_m$, and the parameters of the photosynthesis model of Farquhar *et al.* (1980), the maximum carboxylase activity of Rubisco (V_{cmax}), and the capacity for photosynthetic electron transport (J_{max}) were calculated as in Niinemets *et al.* (1999). In V_{cmax} calculations, the values of Michaelis–Menten constants at 25 °C for CO_2 (K_c) of 274.8 μmol mol^{-1} and for O_2 (K_o) of 414.1 mmol mol^{-1} were from Bernacchi *et al.* (2001).

Characterizing the significance of differences in g_m on photosynthesis

The impact of leaf-to-leaf differences in g_m on photosynthesis depends on the drawdown of CO_2 from substomatal cavities to chloroplasts, i.e. the ratio A/g_m. Actual average CO_2 drawdown from the substomatal cavities to chloroplasts, C_i–C_C, and the ratio of C_C to C_i were calculated for average C_i and C_C values obtained for the ambient CO_2 concentration range of 320–420 μmol mol^{-1} (average 380 μmol mol^{-1}). CO_2 drawdowns from ambient air (C_a) to chloroplasts (C_a–C_C) and from ambient air to substomatal cavities (C_a–C_i) were also calculated.

To reduce the effect of leaf-to-leaf differences in stomatal openness that affect the CO_2 concentration in leaf substomatal cavities (C_i), the fully parameterized model of Farquhar *et al.* (1980) was employed in an iterative manner to calculate the values of A and C_C corresponding to a C_i of 250 μmol mol^{-1} (A_{st} and $C_{C,st}$). From these simulated values, standardized estimates of C_i–C_C and C_C/C_i were derived. Overall, the average (±SE) C_i corresponding to the ambient CO_2 range of 320–420 μmol mol^{-1} was 246.8±4.5 μmol mol^{-1} for all leaves measured, and A_{st} and actual net assimilation rate were strongly correlated (r^2=0.78, P <0.001, n=88), as were the actual and standardized CO_2 drawdowns (r^2=0.39, P <0.001) and C_C/C_i ratios (r^2=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 (r^2; 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 (A_{app}) for a hypothetical situation of $g_m \to \infty$, i.e. $C_i = C_C$. C_C was assumed to be 250 μmol mol^{-1} for all leaves, and A_{app} was calculated. The relative limitation of photosynthesis, Λ_D, due to limited mesophyll diffusion conductance was calculated as

$$\Lambda_D = 1 - \frac{A_{st}}{A_{app}}. \quad (3)$$

Leaf life span and structural analyses

Leaf life span as used in this study refers to the average leaf life span (L_L, years). Previously published values of L_L 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 L_L requires determination of leaf survivorship functions (e.g. Wright and Westoby, 2002; Reich *et al.*, 2004), this information is rarely available, and L_L 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 L_L 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 Imagetool 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 (D_F), dry mass per unit area (M_A), and leaf density ($\mathbf{s}=M_A/T$) 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 Rayment and Higgins (1992) after digestion of samples in a mixture of HNO_3 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 M_A, the correlations between both the area- and mass-based photosynthetic potentials (e.g. V_{cmax}/area and V_{cmax}/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, CO_2 drawdown from substomatal cavities to chloroplasts is the leaf volume-weighted average not the leaf surface-weighted average (Niinemets and Sack, 2006; Niinemets *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 [$y=a+b\mathrm{Log}(x)$ 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_L) 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 ($\mathbf{s}$, ~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.*, 2004*b*), 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

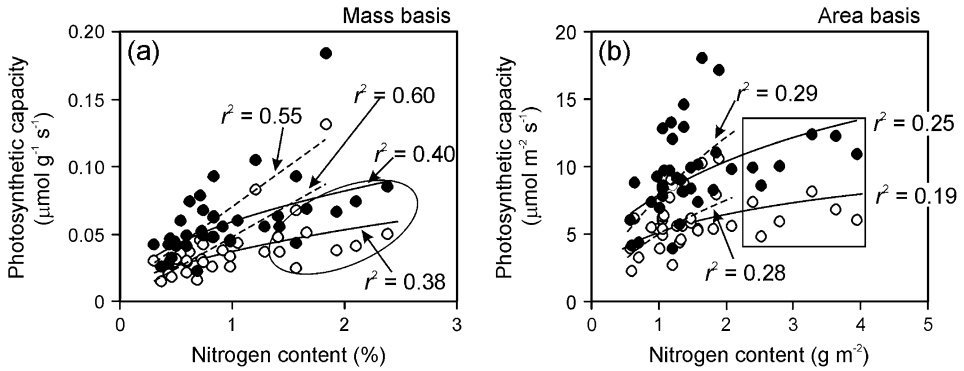

Fig. 1. Correlations of mass- (a) and area-based (b) photosynthetic capacities with nitrogen content in 35 Australian species (see Appendix I for species and Table 1 for sites). Foliage photosynthetic capacity was simulated using the fully parameterized photosynthesis model of Farquhar *et al.* (1980) (V_{cmax}, J_{max}, 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_{st}) or the CO_2 concentration in chloroplasts at 250 μmol mol^{-1} (filled symbols, A_{app}). A_{st} 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 ($C_i=C_C$). 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).

(g_m/mass) 12-fold. g_m/area scaled positively with V_{cmax}/area (Fig. 2a) and J_{max}/area (Fig. 2c), and g_m/mass with V_{cmax}/mass (Fig. 2b) and J_{max}/mass (Fig. 2d), but these correlations were stronger for mass-based relationships. Area-based g_m and stomatal conductance were positively but weakly correlated (r^2=0.23, P <0.005).

Mass-based g_m was negatively associated with M_A (r^2=0.53; Fig. 3a), density (r^2=0.34, P <0.001), thickness (r^2=0.26, P <0.001), and leaf life span (Fig. 3b). g_m/area relationships with leaf life span and structure were much weaker. Although weakly, g_m/area was negatively correlated with L_L (r^2=0.22, P <0.005), but not with M_A, density, and thickness (P >0.1).

CO_2 concentration ratios, drawdowns, and reduction of photosynthesis due to finite g_m

The ratio of CO_2 concentrations in chloroplasts to substomatal cavities, C_C/C_i, varied 1.5-fold among species, while the CO_2 drawdown from substomatal cavities to

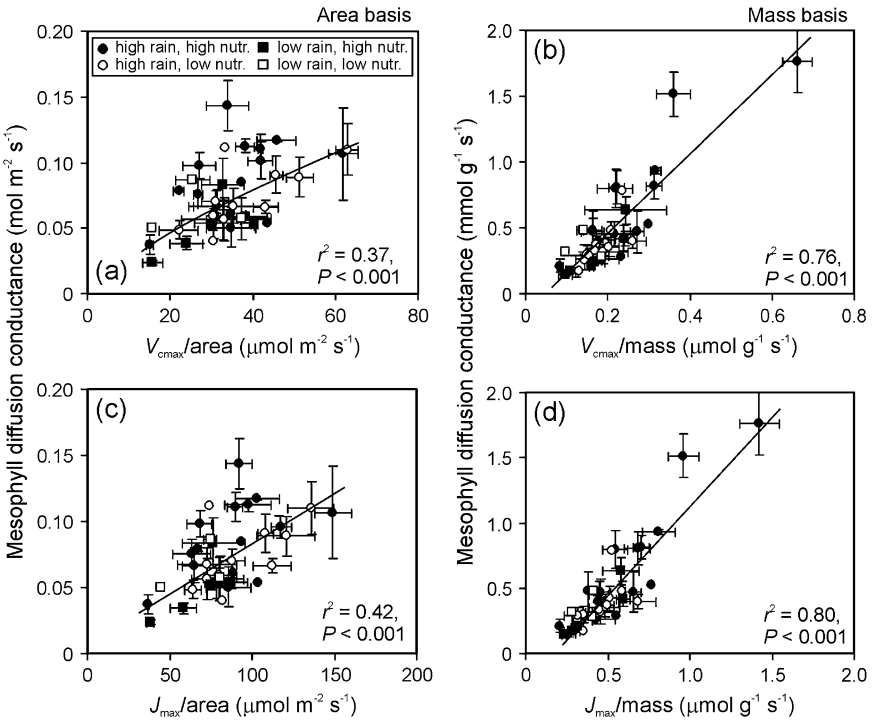

Fig. 2. Internal diffusion conductance to CO_2 (g_m, Eq. 2) per unit area (a, c) and dry mass (b, d) in relation to area- and mass-based maximum Rubisco carboxylase activity, V_{cmax}, (a, b) and the capacity for photosynthetic electron transport, J_{max}, (c, d) in 35 Australian species. The area-based characteristics are the products of mass-based characteristics and leaf dry mass per unit area (M_A). 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 $y=ax^b$ and $y=a+b\text{Log}(x)$ whichever provided the stronger fit (larger r^2). 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.

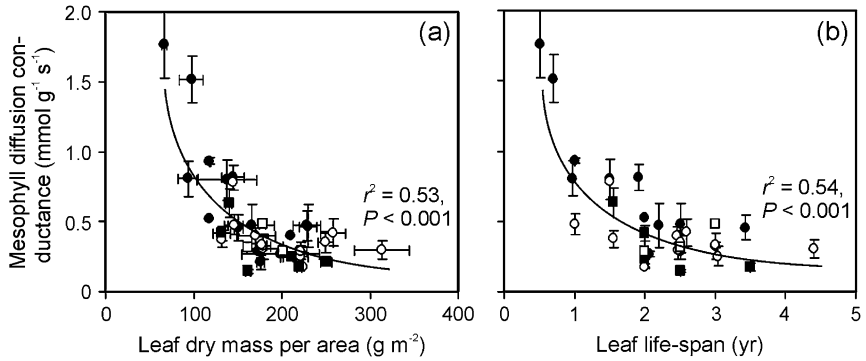

Fig. 3. Mesophyll diffusion conductance per dry mass dependent on leaf dry mass per unit area (M_A) (a) and average foliage life span (b) in 35 Australian species. Data presentation follows Fig. 2. Data were fitted by non-linear regressions in the form of $y=ax^b$.

chloroplasts (C_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. CO_2 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/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/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_i–C_C with net assimilation rate and foliage physiological potentials, V_{cmax} and J_{max}, was poor, with a significant correlation observed only for J_{max}/mass (r^2=0.20, P <0.01 for both the negative correlation with C_i–C_C and the positive correlation with C_C/C_i).

The substomatal to ambient CO_2 concentration ratio, C_i/C_a, 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_i/C_a 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_i/C_a from ~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 μmol mol^{-1}, the correlations of C_C/C_i and C_i–C_C 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 CO_2 drawdown from the ambient air to chloroplasts, C_a–C_C, was still lower in leaves with greater g_m/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 CO_2 concentration due to finite diffusion conductance, Λ_D (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

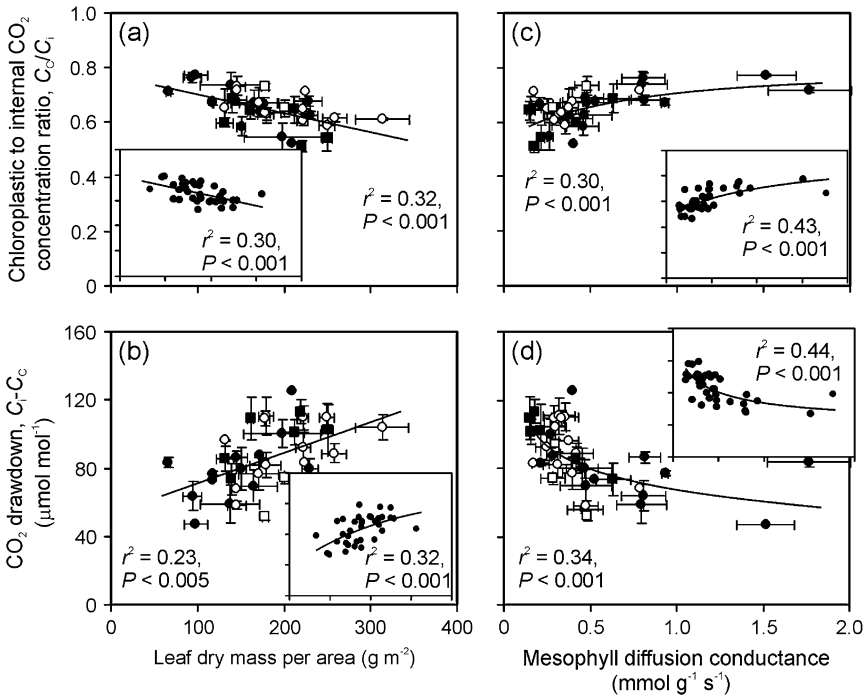

Fig. 4. Chloroplastic (C_C) to internal (substomatal cavities, C_i) CO_2 concentration ratio, C_C/C_i (a, c) and CO_2 drawdown from substomatal cavities to chloroplasts, C_i–C_C (b, d) in relation to M_A (a, b) and g_m/mass (c, d) in 35 Australian species. The values of C_C/C_i and C_i–C_C in the main panels are calculated using the actual C_i and C_C obtained for the ambient CO_2 concentration range of 320–420 μmol mol^{-1}. Insets demonstrate the correlations obtained when C_i was fixed at 250 μmol mol^{-1} for all leaves, and the net assimilation rate (A_{st}) was calculated according to the photosynthesis model of Farquhar *et al.* (1980). Data presentation and fitting are as in Fig. 2.

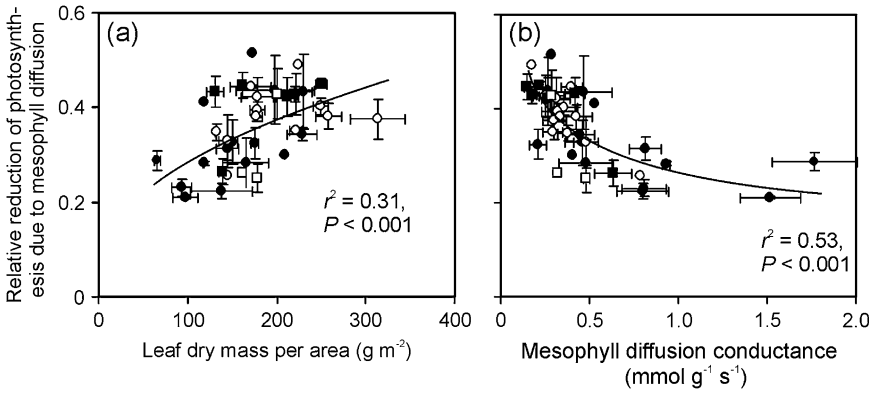

Fig. 5. Relative reduction of the foliage light-saturated photosynthetic rate due to finite diffusion conductance (Eq. 3) dependent on M_A (a) and g_m/mass (b) in 35 Australian species. Data presentation and fitting are as in Fig. 2.

Table 2. Average (± SE) foliage life span (L_L, years), dry mass per unit area (M_A, g m^{-2}), density (s, g cm^{-3}), thickness (T, μm), nitrogen (N_M, %), and phosphorus (P_M, %) contents per dry mass, and average coefficient of variation of these traits (CVs, %) in four contrasting environments*

Resource availability		Variable						
Water	**Nutrients**	L_L†	M_A	s	T	N_M	P_M	**CVs**
High	High	1.63±0.27 a	143±15 a	0.423±0.034 a	343±25 a	1.25±0.16 a	0.0481±0.006 a	38±5 a
High	Low	2.46±0.24 b	201±15 b	0.464±0.029 a	444±26 b	0.82±0.15 a	0.0211±0.0024 b	35±7 a
Low	High	2.31±0.33 a	186±20 a	0.474±0.015 a	392±40 ab	1.06±0.25 a	0.044±0.009 a	33±7 ab
Low	Low	2.50±0.29	180±11	0.459±0.035	396±7	0.56±0.09	0.0275±0.0035	16.3±3.7 b

* Only species sampled from native sites. n=10 for high water/high nutrients, n=13 for high water/low nutrients, n=6 for low water/high nutrients, and n=3 for low water/low nutrients. All average trait values among the sites were compared by ANOVA, while paired t-tests were used to compare the average CV_S corresponding to the six traits. Means with the same letter are not significantly different (P >0.05). The low water/low nutrient site was not included in statistical comparisons due to a limited number of observations.

† Without *Macrozamia* spp. for which no reliable leaf life span data were available.

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_{cmax}/mass, J_{max}/mass, and A_{max}/mass were lower in the high rain/low nutrient site than in the high rain/high nutrient site (Table 3a), reflecting higher M_A in the lower nutrient site (Table 2).

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/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/mass were accompanied by a lower C_C/C_i ratio and greater CO_2 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.*, 2004*b*). Low foliage nutrient contents per mass reflect extreme soil nutrient deficiencies in

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*

(a) Maximum carboxylase activity of Rubisco (V_{cmax}), capacity for photosynthetic electron transport (J_{max}), and light-saturated net assimilation rate (A_{max}) on area (μmol m^{-2} s^{-1}) and mass (μmol g^{-1} s^{-1}) basis

Resource availability		Variable					
Water	**Nutrients**	**V_{cmax}/area**	**V_{cmax}/mass**	**J_{max}/area**	**J_{max}/mass**	**A_{max}/area**	**A_{max}/mass**
High	High	33.7±2.6 a	0.274±0.046 a	83±5 a	0.67±0.10 a	6.5±0.6 a	0.0530±0.010 a
High	Low	37.1±3.0 a	0.186±0.011 b	89±6 a	0.458±0.030 b	6.4±0.5 a	0.0326±0.0023 b
Low	High	28.3±3.4 a	0.170±0.026 ab	65±6 a	0.40±0.06 ab	4.8±0.6 a	0.0267±0.0046 ab
Low	Low	26±6	0.141±0.025	67±11	0.366±0.043	4.8±0.8	0.0262±0.0032

(b) Mesophyll diffusion conductance to CO_2 (g_m) per area (g_m/area, mol m^{-2} s^{-1}), and per mass (g_m/mass, mmol g^{-1} s^{-1}), and the ratios of CO_2 concentrations in substomatal cavities to ambient air (C_i/C_a), chloroplasts (C_C) to that in substomatal cavities (C_C/C_i), CO_2 drawdown C_i–C_C (μmol mol^{-1}), and average coefficients of variation (CV_P) of the 11 traits in (a) and (b)

Resource availability		Variable					
Water	**Nutrients**	**g_m/area**	**g_m/mass**	**C_i/C_a**	**C_C/C_i†**	**C_i–C_C†**	**CV_P**
High	High	0.087±0.010 a	0.74±0.15 a	0.637±0.028 a	0.692±0.018 a	77±5 a	36±6 a
high	Low	0.071±0.006 ab	0.369±0.041 b	0.695±0.019 a	0.630±0.014 b	93±6 b	23.4±2.9 b
Low	High	0.052±0.008 b	0.31±0.8 b	0.654±0.034 a	0.616±0.024 b	96±5 b	30.9±4.5 a
Low	Low	0.065±0.011	0.36±0.06	0.520±0.022	0.70±0.05	76±5	25.4±2.9 ab

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

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.*, 2004*b*). 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 sclerophylly 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 and Sanson, 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.*, 2005*b*), 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

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_2 from substomatal cavities to chloroplasts. It was observed that g_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_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_2 drawdown from the substomatal cavities to chloroplasts is a mesophyll volume-weighted average (Niinemets and Sack, 2006), a stronger correlation between mass-based variables (or more correctly mesophyll mass- or volume-based variables) is expected for data sets with widely varying M_A (provided the variation in M_A reflects modifications in leaf density rather than stacking of mesophyll layers).

Negative correlations between structural traits such as M_A and g_m have been reported previously (e.g. Terashima *et al.*, 2005; Flexas *et al.*, 2008). Again, these relationships are typically expressed on an area basis and are relatively scattered. In our study, we found strong negative correlations between g_m/mass and M_A and foliage longevity (Fig. 3). The correlation of M_A with g_m/mass mostly resulted from the negative scaling of g_m with leaf density rather than with leaf thickness, suggesting that this relationship reflects negative effects of enhanced cell wall thickness on g_m (Terashima *et al.*, 2006). In addition, the overall amount of cell walls and cell wall lignification increases with increasing M_A in Australian species (Groom and Lamont, 1999; Read and Sanson, 2003). The correlations with g_m/area were much weaker, suggesting that g_m/mass is a more appropriate variable to study structural controls on mesophyll diffusion conductance.

So far, mesophyll diffusion limitations had been studied worldwide in only ~120 plant species (Flexas *et al.*, 2008; Warren, 2008). The current study with 35 species of contrasting structure, chemistry, and photosynthetic potentials, covering extremely low values of foliage chemistry and very high values of dry mass per unit area, significantly enlarges the range of data availability. In particular, limited data coverage was available for leaves with M_A values >150 g m^{-2}, and no species with M_A values larger than ~230 g m^{-2} had been measured (Flexas *et al.*, 2008). In fact, in the analysis combining most species measured for g_m so far (Flexas *et al.*, 2008), direct linear extrapolation of the g_m versus M_A relationship suggests that g_m approaches zero at M_A values of ~250 g m^{-2}. The present study found a non-linear dependence of g_m on M_A, demonstrating that the reduction of g_m at the higher end of M_A is asymptotic.

The average g_m/area values of 0.052–0.087 mol m^{-2} s^{-1} observed in the present study (Table 3) are similar to those in other broad-leaved evergreen sclerophyll species (Niinemets *et al.*, 2005*a*, 2006; Flexas *et al.*, 2008). However, average M_A values in this study are somewhat larger than in the other studies with sclerophylls (compare Table 2 and Niinemets *et al.*, 2005*a*, 2006; Flexas *et al.*, 2008). Greater g_m values at a given M_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_A do differ among Australian species (Witkowski and Lamont, 1991). In addition, leaves of a given M_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_m on both a mass and an area basis, and the negative correlation of both g_m/mass and photosynthetic potentials per mass with M_A, the crucial question is to what extent the negative relationship between g_m and M_A results in differences in CO_2 drawdown from the substomatal cavities to chloroplasts (C_i–C_C). It has been suggested previously that the positive correlation, often linear, between photosynthetic capacity and g_m implies that the ratio of the realized net assimilation rate to g_m (A/g_m), i.e. CO_2 drawdown (A/g_m=C_i–C_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_i–C_C scales negatively with g_m and is larger in structurally more robust leaves (Niinemets *et al.*, 2005*a*; 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.*, 2005*a*, 2006; Warren, 2008). Nevertheless, the relative reduction of photosynthesis due to finite diffusion conductance at current ambient CO_2 concentration, Λ_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 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).

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Appendix I. List of studied species with key foliage traits

Species*	Site†	Life span (years)	Dry mass per area (g m^{-2})	Thickness (mm)	Density (g cm^{-3})	N content (%)	P content (%)	Photosynthetic capacity (μmol m^{-2} s^{-1})
Acacia falcata	LRHN	2.0	131.6	0.270	0.486	1.93	0.0531	4.45
Acacia longifolia	MQ	2.0	117.9	0.331	0.357	2.38	0.0411	4.45
Acacia myrtifolia	HRLN	1.5	145.2	0.357	0.407	1.66	0.0148	7.59
Acacia suaveolens	HRLN	2.5	170.9	0.447	0.382	2.11	0.0289	4.93
Angophora hispida	HRLN	2.5	178.9	0.386	0.461	0.60	0.0367	4.50
Astrotricha floccosa	HRHN	0.5	66.1	0.237	0.281	1.84	0.0626	9.19
Banksia aemula	LRLN	3.0	178.1	0.405	0.445	0.50	0.0208	4.50
Banksia integrifolia	MQ	2.2	229.8	0.541	0.439	0.72	0.0412	8.33
Banksia marginata	HRLN	3.0	221.7	0.390	0.575	0.44	0.0109	5.04
Banksia oblongifolia	HRLN	2.6	258.3	0.428	0.605	0.75	0.0219	9.73
Banksia robur	MQ	1.5	138.0	0.499	0.270	0.84	0.0624	5.95
Banksia serrata	MQ	2.5	172.6	0.380	0.453	0.62	0.0431	4.68
Banksia serrata	HRLN	2.5	249.5	0.504	0.496	0.55	0.0250	9.26
Banksia spinulosa	LRHN	3.5	219.8	0.427	0.517	0.46	0.0165	4.39
Breynia oblongifolia	HRHN	0.8	115.9	0.254	0.458	1.61	0.0294	
Corymbia gummifera	HRLN	2.0	224.0	0.405	0.555	0.60	0.0240	3.33
Eriostemon australasius	HRLN	1.0	145.4	0.558	0.265	1.30	0.0111	4.12
Eucalyptus fibrosa	LRHN	2.0	211.6	0.414	0.511	0.99	0.0395	5.02
Eucalyptus haemastoma	LRHN	2.5	161.4	0.382	0.423	0.37	0.0233	2.60
Eucalyptus sclerophylla	LRLN	2.0	201.1	0.383	0.526	0.74	0.0326	4.35
Eucalyptus umbra	HRHN	2.1	198.3	0.406	0.486	0.83	0.0413	5.41
Grevillea speciosa	HRLN	1.6	131.9	0.263	0.506	0.45	0.0128	4.67
Hakea dactyloides	HRLN	4.4	313.4	0.607	0.516	0.43	0.0179	9.26
Lambertia formosa	HRHN	2.5	209.3	0.375	0.559	0.31	0.0183	10.59
Lambertia formosa	HRLN	2.5	221.7	0.398	0.564	0.50	0.0125	7.42
Macadamia ternifolia	MQ	2.5	165.4	0.320	0.515	0.98	0.0648	5.17
Macrozamia communis	HRHN		228.4	0.564	0.405	1.43	0.0641	5.68
Macrozamia spiralis	LRHN		250.4	0.547	0.461	1.58	0.0665	5.50
Notelaea longifolia	HRHN	2.0	176.0	0.322	0.548	0.69	0.0293	3.09
Persoonia lanceolata	HRLN	3.0	178.8	0.506	0.355	0.73	0.0327	5.94
Persoonia laurina	LRLN	2.5	161.7	0.399	0.405	0.44	0.0290	3.11
Persoonia laurina	LRHN	1.6	139.7	0.315	0.446	1.06	0.0652	6.31
Persoonia levis	HRLN	3.0	177.7	0.520	0.342	0.60	0.0247	6.43
Pittosporum undulatum	MQ	1.0	117.9	0.253	0.466	1.58	0.0869	8.66
Polyscias sambucifolia	MQ	0.7	97.6	0.321	0.303	1.22	0.0672	6.75
Syncarpia glomulifera	HRHN	1.9	144.5	0.266	0.542	0.84	0.0351	10.08
Synoum glandulosum	HRHN	1.0	93.9	0.301	0.310	1.41	0.0677	5.00
Xylomelum pyriforme	HRHN	3.4	150.6	0.322	0.470	0.92	0.0336	5.01

* 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.*, 2004*b*), although important discrepancies from worldwide trends can occur within specific parts of the spectrum (Diemer, 1998; Wright *et al.*, 2004*a*). 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 Zamiaceae 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 (**ϛ**) 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 **ϛ** were positively associated with L_L (r^2=0.20, P <0.01 for **ϛ**, and

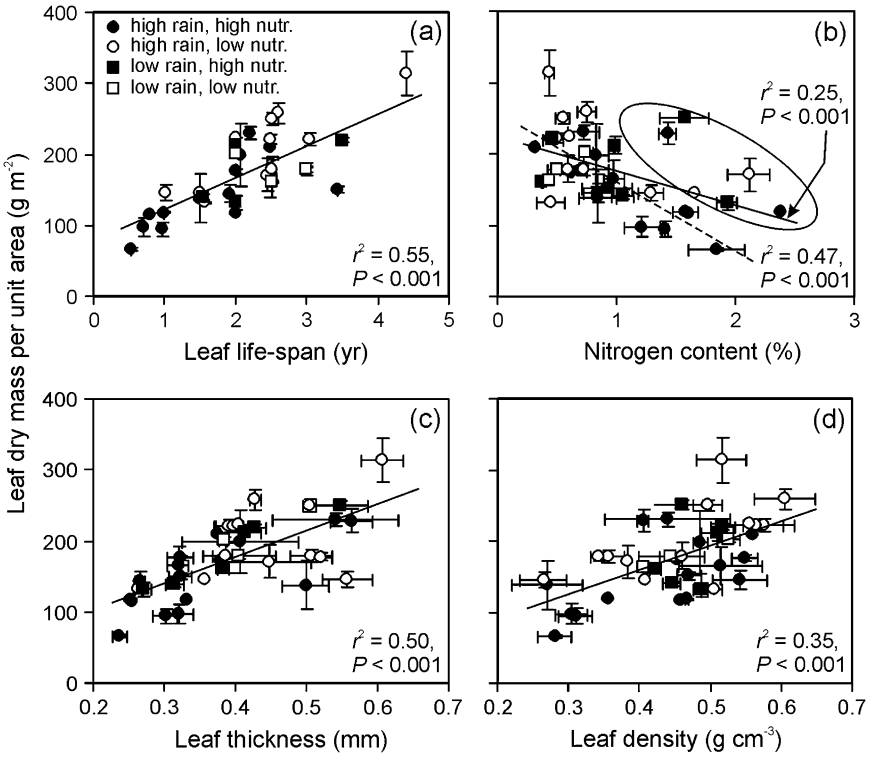

Fig. A1. Dependencies of leaf dry mass per unit area (M_A) on average leaf life span (a), nitrogen content per dry mass (b), leaf thickness (c), and leaf density (d) in 35 Australian tree and shrub species (see Appendix I for the list of species with life span estimates and key structural, chemical, and physiological traits). Each data point corresponds to the average of a given species, and error bars show ±SE. 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). All data pooled across the sites were fitted by linear regressions. In (b), the six nitrogen-fixing species are surrounded by the ellipse, and the regressions with (solid line) and without (dashed line) these data are shown.

r^2=0.28, P <0.001 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 (J_{max}) per unit mass (V_{cmax}/mass and J_{max}/mass) decreased with increasing M_A (Fig. A2a, d) and L_L (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, 2f). 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. A2).

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}/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.*, 2004*b*), 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.*,

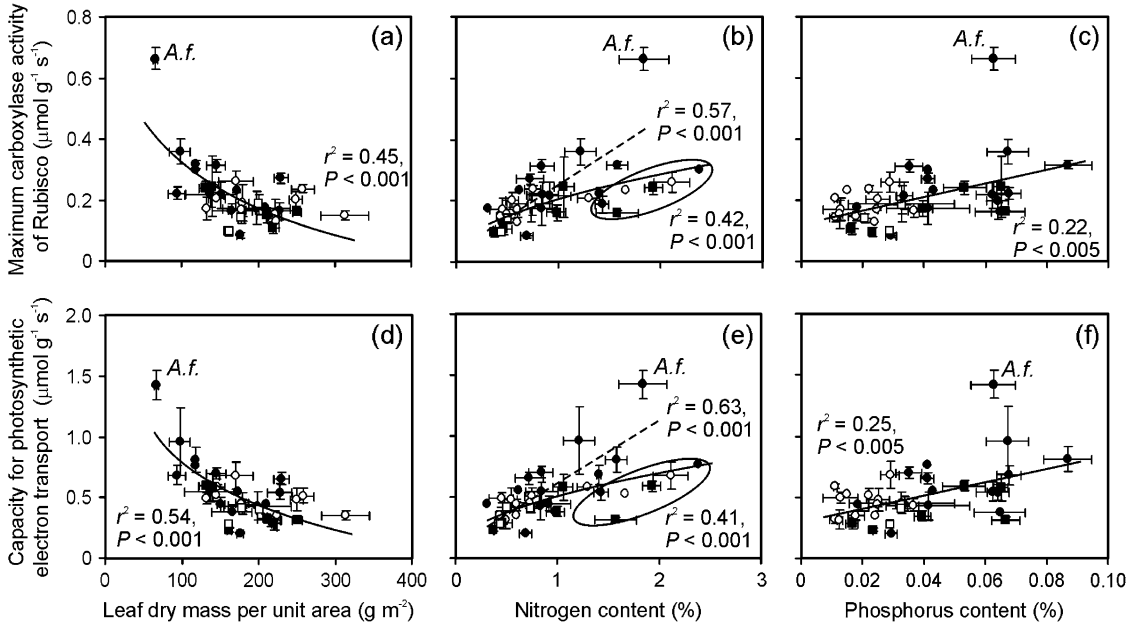

Fig. A2. Correlations of the maximum mass-based Rubisco carboxylase activity (V_{cmax}/mass) (a–c) and the capacity for photosynthetic electron transport (J_{max}/mass) (d–f) with leaf dry mass per area (a, d), nitrogen content per dry mass (b, e), and phosphorus content per dry mass (c, f) in 35 Australian 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 $y=a+b\text{Log}(x)$ 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.

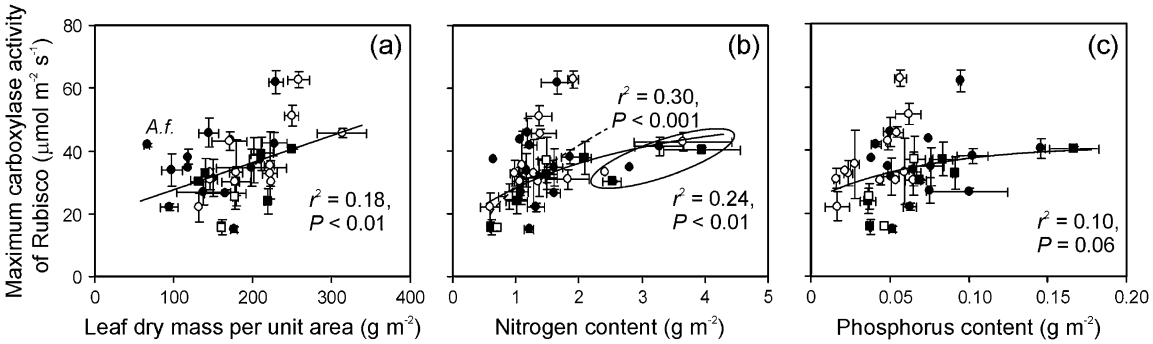

Fig. A3. Relationships of maximum area-based Rubisco carboxylase activity (V_{cmax}/area) with M_A (a), nitrogen (b), and phosphorus (c) contents per area in 35 Australian species. Data presentation and fitting are as in Fig. A2. Fits to the data in (b) are for all species pooled (solid line) and without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

2004*a*) versus up to two orders of magnitude in the GLOPNET data set with worldwide species coverage (Wright *et al.*, 2004*b*). 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).

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