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REVIEW PAPER

Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field

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Abstract

Limited mesophyll diffusion conductance to CO_2 (g_m) can significantly constrain plant photosynthesis, but the extent of g_m-limitation is still imperfectly known. As g_m scales positively with foliage photosynthetic capacity (A), the CO_2 drawdown from substomatal cavities (C_i) to chloroplasts (C_C, C_i–C_C=A/g_m) rather than g_m alone characterizes the mesophyll diffusion limitations of photosynthesis. The dependencies of g_m on A, foliage structure (leaf dry mass per unit area, M_A), and the resulting drawdowns across a dataset of 81 species of contrasting foliage structure and photosynthetic potentials measured under non-stressed conditions were analysed to describe the structure-driven potential photosynthetic limitations due to g_m. Further the effects of key environmental stress factors and leaf and plant developmental alterations on g_m and CO_2 drawdown were evaluated and the implications of varying g_m on foliage photosynthesis in the field were simulated. The meta-analysis demonstrated that g_m of non-stressed leaves was negatively correlated with M_A, and despite the positive relationship between g_m and A, the CO_2 drawdown was larger in leaves with more robust structure. The correlations were stronger with mass-based g_m and A, probably reflecting the circumstance that mesophyll diffusion is a complex three-dimensional process that scales better with mesophyll volume-weighted than with leaf area-weighted traits. The analysis of key environmental stress effects on g_m and CO_2 drawdowns demonstrated that the effect of individual stresses on CO_2 drawdowns varies depending on the stress effects on foliage structure and assimilation rates. Leaf diffusion limitations are larger in non-senescent older leaves and also in senescent leaves, again reflecting more robust leaf structure and/or non-co-ordinated alterations in leaf photosynthesis and g_m. According to simulation analyses, in plants with a larger part of the overall diffusion conductance from the ambient atmosphere to the chloroplasts in the mesophyll, photosynthesis is less sensitive to changes in stomatal conductance. Accordingly, in harsher environments that support vegetation with tougher long-living stress-tolerant leaves with lower g_m, reductions in stomatal conductance that are common during stress periods are expected to alter photosynthesis less than in species where a larger part of the total diffusion limitation is determined by stomata. While structural robustness improves plant performance under environmental stress, low g_m and inherently large CO_2 drawdown in robust leaves limits the photosynthesis of these plants more severely under favourable conditions when stomatal conductance is high. The differences in overall responsiveness to environmental modifications of plants with varying g_m need consideration in current large-scale ecosystem productivity models.

Key words: Diffusion limitations, environmental stress, plant functional types, sclerophylls, stomatal conductance, structure/function relationships.

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Introduction

Plants have evolved different strategies to cope with environmental fluctuations in field environments. While ephemeral short-lived plants such as annual herbs complete the life-cycle during favourable environmental periods, the probability of stress events increases with increasing foliage longevity. Thus, constitutive tolerance of environmental stress becomes increasingly important with increasing longevity. A plethora of structural and physiological modifications occurs with increasing leaf longevity. Specifically, leaf dry mass per unit area (M_A) and foliage density (mass per unit volume) increase as the result of thicker cell walls and a greater fraction of lignified support biomass with increasing leaf longevity (Wright and Cannon, 2001; Wright *et al.*, 2004). These modifications are associated with reduced foliage nitrogen contents (N_M) and photosynthetic potentials (V_{cmax}, J_{max}) per dry mass (Wright *et al.*, 2004). In addition, such profound adjustments in foliage structure can lead to altered CO_2 diffusion conductance from substomatal cavities to chloroplasts, i.e. mesophyll diffusion conductance (g_m). This, in turn, can modify the realized net assimilation rates (A) in leaves with given biochemical potentials of photosynthesis. Thus, g_m can significantly alter the efficiency with which foliage biochemical potentials of photosynthesis are operating under current ambient CO_2 concentrations. Moreover, because finite g_m affects the *calculation* of V_{cmax} and J_{max} (Ethier and Livingston, 2004; Manter and Kerrigan, 2004; Warren, 2008*c*), g_m impairs the *estimated* net assimilation rates using current models of photosynthesis.

Although recent data suggest that g_m can change relatively rapidly in response to fluctuations in environmental drivers (see Flexas *et al.*, 2008, for a review), leaf structure sets the possible upper limit of g_m (Nobel, 1977). As foliage structure strongly adjusts to environmental modifications in time-scales spanning periods of foliage development and overall foliage life span, and plant lifespan (phenotypic adjustments) to evolutionary time-scales (genotypic adjustments), such phenotypic and genotypic alterations modify the response envelopes of g_m versus environmental stress. In the current review analysis, the focus is on such modifications in maximum possible g_m values driven by environmental, ontogenetic, and genetic modifications in foliage structure and the resulting alterations in realized net assimilation rates in leaves acclimated or adapted to different environmental conditions and in leaves and plants of different ontogenetic stage. Determination of such envelope responses requires g_m measurements in non-stressed plants under optimum conditions to avoid artefacts, for instance, due to non-uniform stomatal opening (see Lloyd *et al.*, 1992, for a detailed outline of possible problems). Methods to estimate the maximum values of g_m in stressed leaves have started to be developed (Centritto *et al.*, 2003), but are not routinely used in the studies of photosynthesis. The lack of standardized protocols to avoid artefacts in g_m determinations limits the comparison and interpretation of literature observations but, nevertheless, there is conclusive data on a number of important modifications in maximum g_m values in leaves of differing structure.

In the current analysis, the structural controls on g_m are defined first, and then the changes in g_m in response to various environmental stresses and to leaf development and plant ageing are reviewed. Finally, the influence of different g_m values on the share of diffusion limitations of photosynthesis between mesophyll and stomata and the sensitivity of photosynthesis to fluctuations in stomatal conductance in leaves with differing g_m are analysed.

General correlations of diffusion conductance with foliage structural and physiological traits

Foliage photosynthetic rate, A, and g_m are related as:

$$A = g_m(C_i - C_C) \tag{1}$$

where C_i is the CO_2 concentration in the substomatal cavities and C_C that in the chloroplasts. Thus, the difference in CO_2 concentration, $\Delta_C = C_i - C_C = A/g_m$ is the CO_2 drawdown due to non-infinite g_m. We argue that Δ_C is the appropriate variable gauging whether or whether not leaves with varying g_m are more strongly limited by internal CO_2 diffusion, and should always be estimated to assess the influence of g_m on realized net assimilation rates.

While there is conclusive evidence that g_m values are lower in leaves with more robust structure (for reviews see Evans and Loreto, 2000; Niinemets and Sack, 2006; Warren, 2008*c*), such leaves also possess lower photosynthetic capacity. Thus, strong positive correlations have been found between A and g_m (for reviews see Evans and Loreto, 2000; Flexas *et al.*, 2008). On the basis of simultaneous variation in A and g_m, it has been advocated that the CO_2 drawdown, and, accordingly, the degree to which photosynthesis is limited by g_m does not differ between leaves with contrasting structure (see Evans and Loreto, 2000, for a review). However, recent experimental data and meta-analyses showing a negative correlation between Δ_C and g_m suggest that leaves with greater g_m do have lower mesophyll diffusion limitations of photosynthesis (Niinemets and Sack, 2006; Warren and Adams, 2006; Warren, 2008*c*). As g_m has been observed to scale negatively with leaf dry mass per unit area (M_A) in some studies, larger drawdown in leaves with lower g_m has been suggested to reflect a fundamental structural control on the mesophyll diffusion limitations of photosynthesis (Niinemets and Sack, 2006; Niinemets *et al.*, 2009).

Yet the correlations between g_m, M_A, and Δ_C are often scattered with moderate degrees of explained variance (Flexas *et al.*, 2008). Part of this scatter can be associated with the circumstance that M_A is a composite variable, the product of leaf density (D) and thickness (T) ($M_A = DT$) that can vary independently (Poorter *et al.*, 2009). Although modifications in leaf thickness result in a longer gas-phase diffusion pathway within the leaf, within-leaf gas-phase diffusion is generally a small part of the total diffusion pathway (Parkhurst and Mott, 1990; Terashima *et al.*, 1995). At the same time, increased leaf thickness is commonly associated with a greater number of mesophyll cell layers. A larger amount of mesophyll per unit leaf area implies a larger area of mesophyll exposed to intercellular air space (S_m), and thus greater S_m to total leaf surface (S) area ratio (S_m/S) (Nobel, 1976, 1977). Provided that the number of chloroplasts is similar in mesophyll cells, larger S_m/S also implies a greater chloroplast exposed surface area to total surface area ratio (S_C/S) (Terashima *et al.*, 2005, 2006). As larger S_C/S implies more parallel pathways for CO_2 liquid-phase diffusion, g_m scales positively with S_C/S (Nobel, 1991). Thus, g_m may actually increase with increasing T (Terashima *et al.*, 2001).

In contrast, increases in leaf density are associated with reduced gas-phase volume, and smaller and more densely packed mesophyll cells with thicker cell walls (for reviews see Niinemets, 1999; Niinemets and Sack, 2006). Such modifications will reduce the liquid-phase diffusion conductance and g_m (Terashima *et al.*, 2005; Evans *et al.*, 2009). In fact, it has been demonstrated that the correlations are stronger with a leaf dry to fresh mass ratio that is a substitute of D than with M_A (Niinemets *et al.*, 2005), supporting the suggestion that M_A as an integral variable may not be the best to describe the diffusional limits of photosynthesis.

A further reason for the poor correlations can be the conceptual difficulty in expressing the mesophyll diffusion conductance. Diffusion inside the leaves is inherently a three-dimensional phenomenon (see Parkhurst, 1994, for extensive discussion). CO_2 drawdown from the internal airspace to chloroplasts is a mesophyll-volume weighted average, and thus, it should more strongly scale with overall photosynthetic tissue volume than with area, i.e. with g_m expressed per unit leaf volume or leaf mass (g_m/M_A) than with g_m/area (Niinemets *et al.*, 2005; Niinemets and Sack, 2006). The importance of distinguishing between area- and mass-based characteristics becomes particularly evident when leaves with widely varying S_C/S, that also differ in M_A, are compared (Terashima *et al.*, 2005; Evans *et al.*, 2009). g_m/mass as a physical quantity may seem awkward, but not if one considers it as a measure of the extent to which the photosynthesis of average mesophyll cells is limited by mesophyll diffusion conductance. Equation 1 can be expressed as:

$$A/\text{mass} = g_m/\text{mass}(C_i - C_C) \qquad (2)$$

where A/area and A/mass are related as $A/\text{area} = M_A A/\text{mass}$. Modifications in both g_m/area and A/area can occur because of modifications in M_A without any significant alteration of photosynthetic potential of single mesophyll cells, for instance, due to stacking of mesophyll biomass as the result of increases in leaf thickness and the number of mesophyll layers (see the growth light effects on g_m in this article). As a mass basis characterizes the average photosynthetic capacity and average diffusion limitations of leaf cells, the use of mass-based characteristics can provide additional insight into the biochemical and mesophyll diffusion limitations of photosynthesis. Ideally, the appropriate expression basis is mass or volume of leaf mesophyll rather than total leaf mass, but mesophyll volume is rarely estimated in leaf structure–function studies. Further A/mass rather than A/area is the key characteristic scaling (negatively) with leaf longevity and M_A in the worldwide leaf economics spectrum (Wright *et al.*, 2004). Thus, g_m/mass is the characteristic that should be included in the leaf structure/function analyses.

Finally, the extensive meta-analyses conducted so far have pulled together data from experiments under a variety of treatments (Warren and Adams, 2006; Flexas *et al.*, 2008; Warren, 2008*c*). Given that a series of environmental stresses can result in rapid reductions in g_m in leaves with a similar structure or modifications in structure without alteration of CO_2 drawdown (for instance, during shade adaptation), stress probably partly impairs structure and mesophyll diffusion conductance. Thus, seeking correlations between g_m and foliage structural and physiological traits should be limited to maximum g_m values measured under non-stressed conditions.

To test conclusively for g_m and Δ_C versus structure and photosynthetic capacity relationships, a meta-analysis was conducted based on 60 individual studies reporting data for 81 species, i.e. *c.* 30% more studies for pertinent relationships (Figs 1, 2) than used in the previous analyses (Niinemets and Sack, 2006; Flexas *et al.*, 2008; Warren, 2008*c*) (see Table 1 for a species list with corresponding references and the Supplementary Appendix at *JXB* online for species average structural and physiological traits for each study). In addition to including more information, our analysis differs from previous analyses (Flexas *et al.*, 2008; Warren, 2008*c*) by only including experiments under non-stressed conditions, i.e. typically the control treatment (high light and water availability, low altitude) to avoid confounding the structural and physiological controls with experimental treatment effects. Only measurements under ambient CO_2 (335–410 μmol mol^{-1}, average $\pm$SE=367$\pm$0.9 μmol mol^{-1}) were included in the analysis. Altogether 271 observations were available for area-based characteristics and 91 for M_A and mass-based characteristics.

Area-based relationships demonstrated a significant curvilinear reduction of g_m/area with M_A (Fig. 1a), and positive relationship with A/area (Fig. 1b). A wide range of values of g_m/area corresponded to any given value of A/area, and thus, the drawdown due to g_m, $A/g_m = C_i - C_C$, was not constant for leaves differing in M_A and g_m. In fact, Δ_C was negatively associated with g_m/area. Although, the drawdown is expected to be positively linked to photosynthetic capacity, there was a weak negative relationship between A and $C_i - C_C$ (Fig. 1d). These results agree with previous summary analyses (Warren and Adams, 2006; Flexas *et al.*, 2008; Warren, 2008*c*), except for a somewhat higher degree of explained variance (r^2) in g_m/area versus

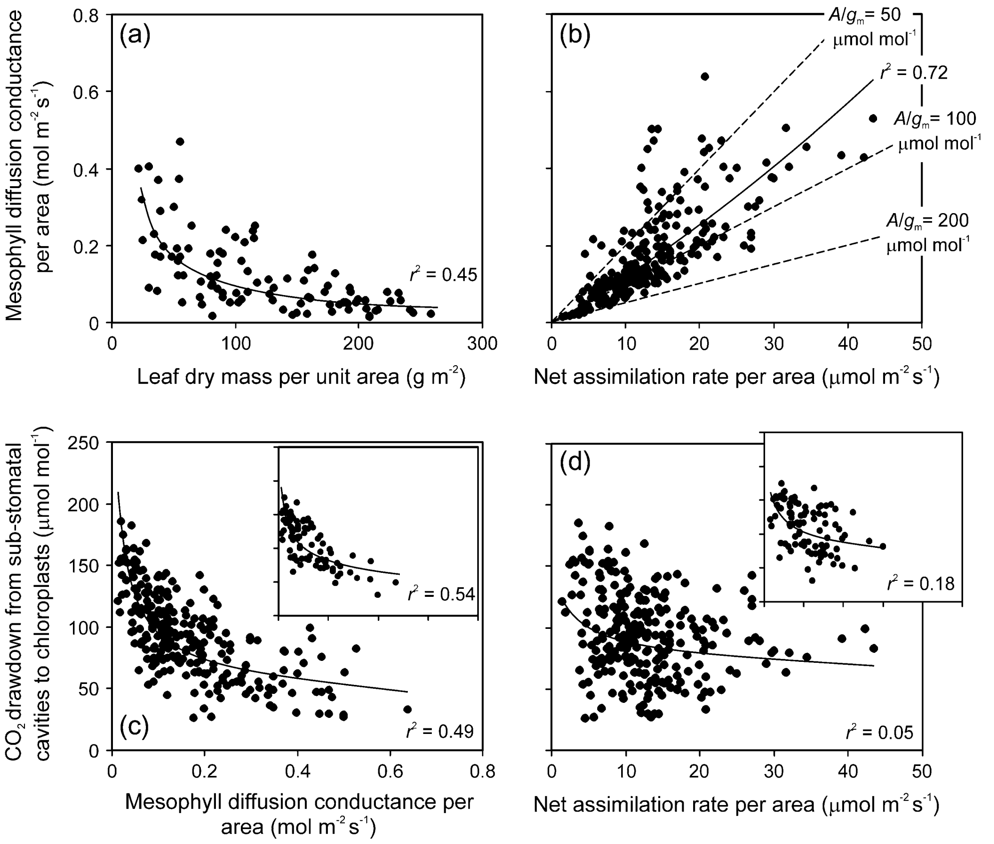

Fig. 1. Correlations between mesophyll diffusion conductance per leaf area (g_m/area) with leaf dry mass per unit area (M_A) (a), and light-saturated net assimilation rate per unit leaf area (photosynthetic capacity, A/area) (b) and corresponding relationships of CO$_2$ drawdown from substomatal cavities (C_i) to chloroplasts (C_C) versus g_m/area (c) and A/area (d). The data were fitted by non-linear regressions in the form of $y=ax^b$, and all are significant at P <0.001. n=271 for (b), (c), and (d). As M_A values were not available in all cases, n=96 for (a). Insets in (c) and (d) demonstrate the relationship with the truncated dataset corresponding to (a). For (b), r^2 for the truncated dataset was 0.85. As the drawdown, C_i–C_C is equal to A/g_m (equation 1), a perfect correlation between A versus g_m would imply a constant CO$_2$ drawdown. Slopes corresponding to constant CO$_2$ drawdowns of 50, 100, and 200 μmol mol^{-1} are shown by dashed lines in (b). The dataset used is based on 60 individual studies (see Table 1 for species list with corresponding references) reporting data for 81 species. Only experiments under non-stressed conditions, i.e. typically the control treatment (high light and water availability, low altitude) were included in the analysis.

M_A and g_m/area versus Δ_C relationships due to the consideration of non-stressed plants only.

Mass-based relationships were broadly similar (Fig. 2), but r^2 values were, in all cases, somewhat larger (cf. Figs 1 and 2). Although the sample size was smaller for the mass-based relationships, r^2 values for the area-based relations in a subset of data used for developing mass-based relations were still lower (inset in Fig. 1c for Δ_C versus g_m/area, and in Fig. 1d for Δ_C versus A/area; r^2=0.85 for a truncated dataset of A/area versus g_m/area relationship in Fig. 1b using only the data points having M_A values).

The drawdown of CO$_2$, C_i–C_C, was larger in leaves with greater M_A (Fig. 3), demonstrating that photosynthesis in leaves with more robust structure is more strongly limited by g_m. The drawdown from ambient air (C_a) to substomatal cavities was also positively correlated with M_A, but the correlation was weak (r^2=0.12, P <0.001). The overall drawdown from the ambient atmosphere to the chloroplasts, C_a–C_C scaled positively with M_A as well (r^2=0.17, P <0.001). M_A was negatively correlated with both A/area and A/mass (for non-linear regressions in the form of $y=ax^b$, r^2=0.32 for A/area and r^2=0.79 for A/mass, P <0.001 for both) agreeing with worldwide patterns (Wright *et al.*, 2004). These negative correlations possibly explain the unexpected weak negative scaling of CO$_2$ drawdown with photosynthetic capacity (Figs 1d, 2d), i.e. lower mesophyll diffusion limitation of photosynthesis in physiologically more active leaves with lower M_A that also possessed a larger g_m.

While area- and mass-based relationships were qualitatively similar, the higher degree of explained variance in mass-based relationships suggests that the mass basis provides a more effective estimate of internal CO$_2$ concentration, as previously suggested for datasets with lower species coverage (Niinemets *et al.*, 2005; Niinemets and Sack, 2006). Nevertheless, the correlation between M_A and Δ_C was quite scattered, possibly reflecting the complex nature of M_A as a leaf parameter depending on both thickness and density. Clearly, studies dissecting M_A into thickness and density are needed to gain further insight into

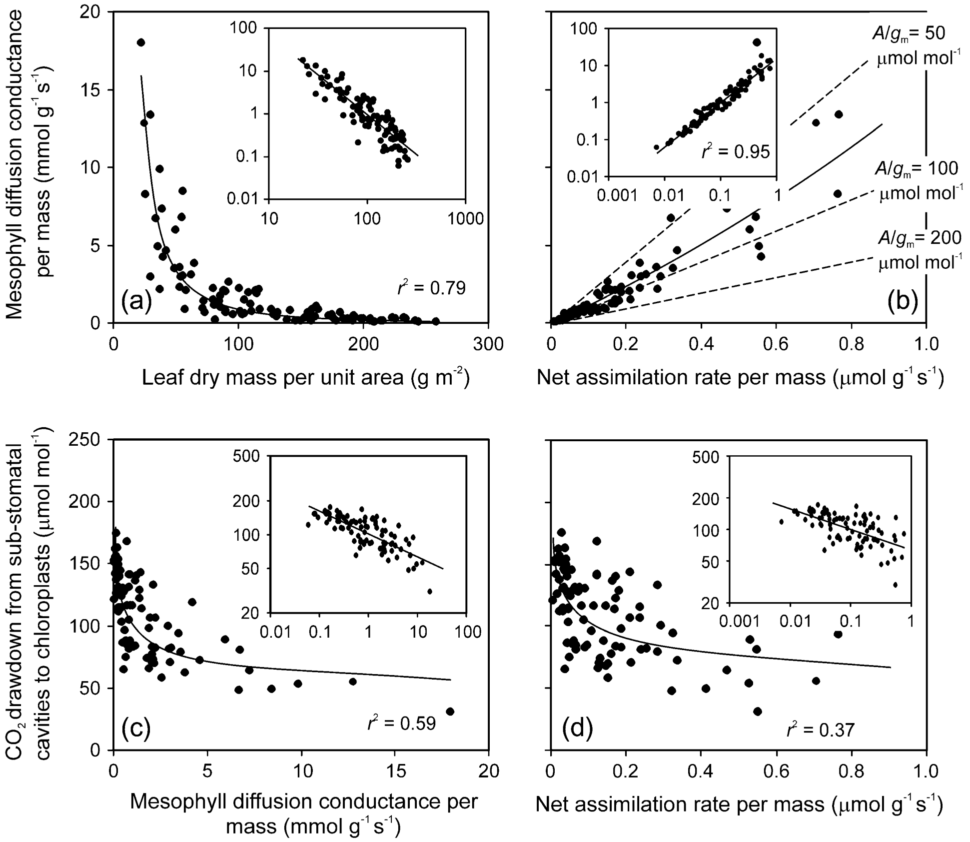

Fig. 2. Relationships between mass-based diffusion conductance (g_m/mass) with M_A (a) and A/mass (b), and the corresponding CO$_2$ drawdowns in relation to g_m/mass (c) and A/mass (d) for the same dataset as in Fig. 1. As mass-based characteristics could only be calculated when M_A was available, n=96 in all cases. The insets demonstrate the correlations in log–log axes. As in Fig. 1b, the dashed lines show the slopes corresponding to constant CO$_2$ drawdowns 50, 100, and 200 μmol mol^{-1}. Data fitting as in Fig. 1 (P <0.001 for all).

the structural controls of g_m, but the available evidence suggests that such a general scaling is important for plant leaves widely varying in structural traits.

Implications for structural scaling of g_m

Foliage structure as characterized by M_A and other key structural traits varies greatly among the major plant functional types, and this variation is correlated with different foliage longevities and different tolerance of environmental stresses. Average values of M_A increase in the following manner: annual herbs <perennial herbs <deciduous trees <evergreen broad-leaved trees <evergreen sclerophylls <evergreen conifers (Poorter *et al.*, 2009). This ranking matches the order of decreasing g_m among plant functional groups (Flexas *et al.*, 2008). This coupled variation is significant, as modification in the plant functional type spectrum forms a key difference among ecosystems. For instance, mesic temperate ecosystems in the Northern hemisphere are dominated by broad-leaved winter-deciduous species and/or conifers and by broad-leaved evergreens in warm temperate and oceanic temperate forests in East Asia and in the Southern hemisphere, while extreme dry environments are characterized by succulents or drought-deciduous species. Mediterranean environments with highly seasonal precipitation are semi-arid environments still supporting evergreen broad-leaved vegetation, and they are characterized by species with very rigid cell walls and large mesophyll diffusion limitations of photosynthesis (Galmés *et al.*, 2007*c*).

Such changes in the functional type spectrum can have important implications for the share of photosynthetic limitations between enzyme activities and diffusion. Worldwide, sites with increasingly lower water availability support species with denser leaves and larger M_A (Niinemets, 2001; Wright *et al.*, 2005). As g_m is linked to leaf structure, the correlations of leaf structure with site climate are of major significance for the prediction of global photosynthetic production. Finding positive correlations between M_A and CO$_2$ drawdown due to limited g_m suggests that photosynthesis in plants growing in harsher environments and having structurally more robust leaves is more strongly controlled by limited diffusion conductance than photosynthesis in species from more favourable environments, for example, Mediterranean evergreen versus deciduous broad-leaved temperate species (Flexas *et al.*, 2008; Warren, 2008*c*). Strong negative relationships between A/mass and M_A are

Table 1. List of species (81 different taxonomic entries) and corresponding references (60 studies) used in the meta-analysis exploring the relationships between leaf structure, mesophyll diffusion conductance and photosynthetic capacity (Figs 1–3)

Average foliage structural and physiological traits for each species in a given study are reported in the Supplementary Appendix at *JXB* online.

Species[a]	Reference	Life form
Acer mono	Hanba *et al.*, 2001	Deciduous tree
Acer mono	Hanba *et al.,* 2002	Deciduous tree
Acer palmatum	Hanba *et al.,* 2002	Deciduous tree
Acer rufinerve	Hanba *et al.*, 2002	Deciduous tree
Alnus japonica	Hanba *et al.*, 2001	Deciduous tree
Arabidopsis thaliana	Tholen *et al.*, 2008	Herb
Arabidopsis thaliana	Flexas et al., 2007*b*	Herb
Arbutus unedo	Loreto et al., 1992	Evergreen shrub
Beta maritima subsp. *marcosii*	Galmés *et al.*, 2007*c*	Herb
Beta maritima subsp. *maritima*	Galmés *et al.*, 2007*c*	Herb
Beta vulgaris	Loreto *et al.*, 1992	Herb
Bruguiera parviflora	Parida *et al.*, 2004	Evergreen tree
Camellia japonica	Hanba *et al.*, 1999	Evergreen shrub
Capsicum annuum	Delfine *et al.*, 2001	Herb
Castanea sativa	Epron *et al.*, 1995	Deciduous tree
Castanea sativa	Lauteri *et al.*, 1997	Deciduous tree
Castanopsis sieboldii	Hanba *et al.*, 1999	Evergreen tree
Castanopsis sieboldii	Miyazawa and Terashima, 2001	Evergreen tree
Cinnamomum camphora	Hanba *et al.*, 1999	Evergreen tree
Cistus albidus	Galmés *et al.*, 2007*c*	Evergreen shrub
Citrus limon	Lloyd *et al.*, 1992; Syvertsen *et al.*, 1995	Evergreen tree
Citrus paradisi	Lloyd et al., 1992; Syvertsen et al., 1995	Evergreen tree
Cucumis sativus	Juszczuk *et al.*, 2007	Herb
Cucumis sativus	Loreto *et al.*, 1992	Herb
Cypripedium flavum	Zhang *et al.*, 2008	Herb
Dacrydium cupressinum	DeLucia *et al.*, 2003	Evergreen conifer
Digitalis minor var. *minor*	Galmés *et al.*, 2007*b*	Herb
Digitalis minor var. *palaui*	Galmés *et al.*, 2007*b*	Herb
Diplotaxis ibicensis	Galmés *et al.*, 2007*c*	Herb
Eucalyptus blakelyi	von Caemmerer and Evans, 1991	Evergreen tree
Eucalyptus globulus	Loreto *et al.*, 1992	Evergreen tree
Eucalyptus globulus	Warren, 2004	Evergreen tree
Eucalyptus regnans	Warren, 2008b	Evergreen tree
Fagus sylvatica	Epron *et al.*, 1995	Deciduous tree
Fagus sylvatica	Warren *et al.*, 2007	Deciduous tree
Fraxinus angustifolia	Grassi and Magnani, 2005	Deciduous tree
Glycine max	Bernacchi *et al.*, 2005	Herb
Glycine max	Flexas *et al.*, 2006*a*	Herb
Glycine max	Gillon and Yakir, 2000	Herb
Gossypium hirsutum	Ennahli and Earl, 2005	Evergreen shrub
Hedera helix	Loreto *et al.*, 1992	Evergreen vine
Hypericum balearicum	Galmés et al., 2007*c*	Herb
Juglans nigra×regia	Piel *et al.*, 2002	Deciduous tree
Laurus nobilis	Niinemets *et al.*, 2005	Evergreen tree
Ligustrum lucidum	Hanba *et al.*, 1999	Evergreen tree
Limonium gibertii	Flexas *et al.*, 2007*a*	Herb
Limonium gibertii	Galmés *et al.*, 2007*c*	Herb
Limonium magallufianum	Galmés *et al.*, 2007*c*	Herb
Lysimachia minoricensis	Galmés *et al.*, 2007*a*	Herb
Macadamia integrifolia	Lloyd *et al.*, 1992; Syvertsen *et al.*, 1995	Evergreen tree
Mangifera indica	Urban *et al.*, 2008	Evergreen tree
Mentha spicata	Delfine *et al.*, 2005	Herb
Metrosideros umbellata	DeLucia *et al.*, 2003	Evergreen tree
Nerium oleander	Loreto *et al.*, 1992	Evergreen tree
Nicotiana sylvestris	Priault *et al.*, 2006	Herb
Nicotiana tabacum	Miyazawa *et al.*, 2008	Herb
Nicotiana tabacum	Evans *et al.*, 1994	Herb
Nicotiana tabacum	Galmés *et al.*, 2006	Herb
Nicotiana tabacum	Gillon and Yakir, 2000	Herb
Nicotiana tabacum	von Caemmerer and Evans, 1991	Herb
Nicotiana tabacum	Flexas et al., 2006*b*	Herb
Nicotiana tabacum	Flexas et al., 2006*a*	Herb
Olea europaea	Diaz-Espejo et al., 2007	Evergreen tree
Olea europaea	Loreto et al., 2003	Evergreen tree
Olea europaea	Marchi et al., 2008	Evergreen tree
Olea europaea	Centritto et al., 2003	Evergreen tree
Olea europaea	Bongi and Loreto, 1989	Evergreen tree
Olea europaea	Niinemets et al., 2005	Evergreen tree
Oryza sativa	Hanba *et al.*, 2004	Grass
Oryza sativa	Sasaki *et al.*, 1996	Grass
Oryza sativa	von Caemmerer and Evans, 1991	Grass
Phaseolus vulgaris	Flowers *et al.,* 2007	Herb
Phaseolus vulgaris	Bota *et al.*, 2004	Herb
Phaseolus vulgaris	DeLucia *et al.*, 2003	Herb
Phaseolus vulgaris	von Caemmerer and Evans, 1991	Herb
Phaseolus vulgaris	Warren, 2008b	Herb
Phlomis italica	Galmés *et al.*, 2007*c*	Herb
Phragmites australis	Antonielli *et al.*, 2002	Grass
Pinus radiata	DeLucia *et al.*, 2003	Evergreen conifer
Pistacia lentiscus	Galmés *et al.*, 2007*c*	Evergreen shrub
Polygonum cuspidatum	Kogami *et al.*, 2001	Herb
Populus maximowiczii	Hanba *et al.*, 2001	Deciduous tree
Populus sp.	Roupsard *et al.*, 1996	Deciduous tree
Prumnopitys ferruginea	DeLucia *et al.*, 2003	Evergreen conifer
Prunus persica	Marchi *et al.*, 2008	Deciduous tree
Prunus persica	Lloyd et al., 1992; Syvertsen et al., 1995	Deciduous tree
Pseudotsuga menziesii	Ethier *et al.*, 2006	Evergreen conifer
Pseudotsuga menziesii	Warren *et al.*, 2004	Evergreen conifer
Quercus canariensis	Warren and Dreyer, 2006	Deciduous tree
Quercus glauca	Hanba *et al.*, 1999	Evergreen tree
Quercus ilex	Loreto *et al.*, 1992	Evergreen tree
Quercus ilex	Niinemets *et al.*, 2005	Evergreen tree
Quercus ilex	Roupsard *et al.*, 1996	Evergreen tree
Quercus petraea	Roupsard *et al.*, 1996	Deciduous tree
Quercus phillyraeoides	Hanba *et al.*, 1999	Evergreen tree
Quercus robur	Gillon and Yakir, 2000	Deciduous tree
Quercus robur	Grassi and Magnani, 2005	Deciduous tree

Table 1. (*Continued*)

Species[a]	**Reference**	**Life form**
Quercus robur	Roupsard *et al.*, 1996	Deciduous tree
Quercus rubra	Loreto *et al.*, 1992	Deciduous tree
Raphanus sativus	von Caemmerer and Evans, 1991	Herb
Rhamnus alaternus	Bota *et al.*, 2004	Evergreen shrub
Rhamnus ludovici-salvatoris	Bota *et al.*, 2004	Evergreen shrub
Rosmarinus officinalis	Delfine *et al.*, 2005	Evergreen shrub
Solanum lycopersicum	Warren, 2008b	Herb
Spinacia oleracea	Delfine *et al.*, 1999	Herb
Spinacia oleracea	Yamori *et al.*, 2006	Herb
Triticum aestivum	von Caemmerer and Evans, 1991	Grass
Triticum durum	Loreto *et al.*, 1994	Grass
Triticum spp.	Loreto *et al.*, 1992	Grass
Vicia faba	Loreto *et al.*, 1992	Herb
Vitis berlandieri x V. rupestris	Flexas *et al.*, 2007*a*	Deciduous vine
Vitis vinifera	Düring, 2003	Deciduous vine
Vitis vinifera	Flexas *et al.*, 2002	Deciduous vine
Vitis vinifera	Diaz-Espejo, unpublished data	Deciduous vine
Vitis vinifera×*V. berlandieri*	Fila *et al.*, 2006	Deciduous vine
Weinmannia racemosa	DeLucia *et al.*, 2003	Evergreen tree
Xanthium strumarium	Loreto *et al.*, 1992	Herb

[a] Species taxonomy follows that used in the original studies.

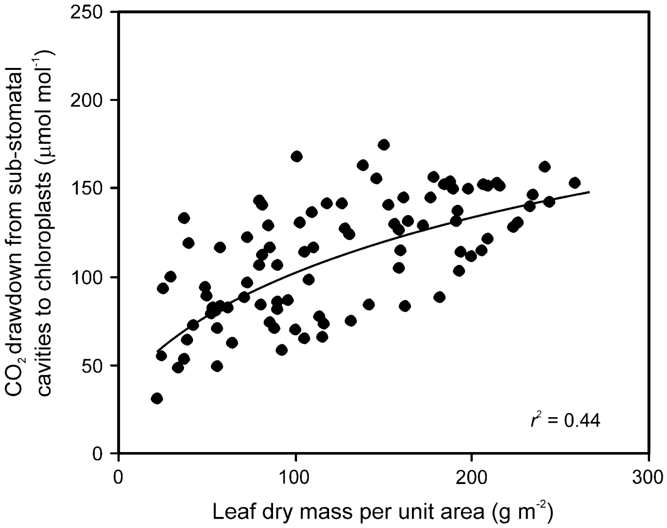

Fig. 3. Dependence of CO_2 drawdown from substomatal cavities to chloroplasts on M_A for the same dataset as in Fig. 1 (n=96). Data fitting as in Fig. 1 (P <0.001).

observed worldwide, reflecting a global convergence in leaf trait relationships (Wright *et al.*, 2004). These relationships so far ignore the role of finite g_m in affecting any given value of A/mass. As A/mass of leaves with certain investments in photosynthetic proteins depends on the extent to which diffusion limits photosynthesis, the mesophyll diffusion limitation is an important factor shaping the global economic spectrum of leaf photosynthetic differentiation (Wright *et al.*, 2004).

Effects of key environmental stresses on intraspecific variability in mesophyll diffusion conductance

Apart from constitutive differences in diffusion limitations among plant species with differing structure and photosynthetic potentials, plants have a large phenotypic plasticity to various environmental stress factors that can importantly modify A versus g_m and g_m versus structure relationships. In the following, the responses of g_m to key environmental drivers of worldwide significance are analysed.

Responses to limited water availabilities and salt stress

Differences in water availability is the key climatic factor and environmental stress worldwide with tremendous spatial variability and temporal fluctuations occurring during the growing season, between the seasons and years. Water stress can induce a rapid reduction in g_m, for example, after the addition of polyethylene glycol (PEG) (Warren *et al.*, 2004) or abscisic acid (Flexas *et al.*, 2006*a*) to plants in hydroponic solution, or during rapid dehydration in severed leaves (Flexas *et al.*, 2008). Such alterations are compatible with the rapid hardening of cell walls immediately after stress application, possibly mediated by hydraulic signals (Chazen and Neumann, 1994; Chazen *et al.*, 1995), but may also depend on aquaporin-mediated modifications in the permeability of membranes in the diffusion pathway (Miyazawa *et al.*, 2008). These rapid reductions in g_m can be partly alleviated after stomatal opening, for instance, by the conditioning of plants with constrained water status in the atmosphere of low atmospheric CO_2 that is known to promote stomatal opening (Centritto *et al.*, 2003). Longer-term experimental studies have shown reductions in g_m in leaves during cycles of drought of varying length and severity (Fig. 4; Flexas *et al.*, 2002; Warren *et al.*, 2004; Monti *et al.*, 2006; Galmés *et al.*, 2007*c*; Warren, 2008*b*; Peeva and Cornic, 2009). Currently, the extent to which drought-induced changes in g_m reflect physiological controls, alterations in foliage photosynthetic potentials (in line with A versus g_m relationship), or modifications in leaf structure is not known. This lack of general consensus is partly driven by widely differing severity and duration of imposed water stress conditions, as well as overall difficulties in reliable estimation of CO_2 concentrations in substomatal cavities due to possible non-uniform stomatal closure in droughted plants (Downton *et al.*, 1988; van Kraalingen, 1990), and thus, difficulties in the estimation of the degree of stomatal versus biochemical reduction of photosynthesis in drought-stressed plants.

A meta-analysis compiling data from ten studies for 21 species from a variety of experiments suggests that A versus g_m change simultaneously in droughted plants (Fig. 4). This partly reflects reduced stomatal conductance and overall lower CO_2 concentrations, but photosynthetic capacity can also change, in particular, after prolonged drought (Medrano *et al.*, 2002). As a result of such simultaneous changes in A and g_m, the drawdown of CO_2 from

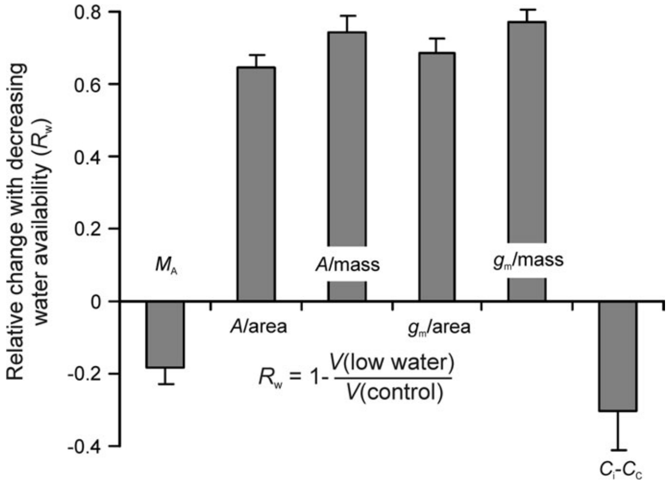

Fig. 4. Changes in leaf dry mass per unit area (M_A), area- and mass-based leaf photosynthetic capacity (light-saturated net assimilation rate) and mesophyll diffusion conductance and respective CO_2 drawdown from substomatal cavities to chloroplasts due to limited water availability. Relative change (R_W) in each of these characteristics (where *V* stands for the given trait) was calculated as shown in the figure. The averages ±SE are based on 21 herb, shrub and tree species of contrasting foliage structure investigated in 10 individual studies: *Olea europaea* (Bongi and Loreto, 1989), *Mentha spicata* and *Rosmarinus officinalis* (Delfine *et al.*, 2005), *Spinacia oleracea* (Delfine *et al.*, 1999), *Vitis vinifera* cv. Tempranillo (Flexas *et al.*, 2002), *Beta maritima* subsp. *maritima*, *Cistus albidus*, *Diplotaxis ibicensis*, *Hypericum balearicum*, *Lavatera maritima*, *Limonium gibertii*, *Limonium magallufianum*, *Phlomis italica*, *Pistacia lentiscus*, *Lysimachia minoricensis* (Galmés *et al.*, 2007*c*), *Nicotiana tabacum* (Galmés *et al.*, 2006; Miyazawa *et al.*, 2008), *Bruguiera parviflora* (Parida *et al.*, 2004), *Pseudotsuga menziesii* (Warren *et al.*, 2004), *Eucalyptus regnans*, *Phaseolus vulgaris*, *Solanum esculentum* (Warren, 2008*b*). The change in plant water status was obtained by altering soil water availability in all studies except those by Bongi and Loreto (1989) and Delfine *et al.* (1999), where plant water status changed due to imposed salinity stress. The duration and severity of water stress varied among the studies. Area-based values are the products of mass-based values and M_A. For mass-based characteristics and M_A, n=11.

substomatal cavities to chloroplasts changes less than either A or g_m (Fig. 4). Nevertheless, C_i–C_C was larger in water-limited plants in the majority of cases (16 out of 23, and the average relative difference in C_i–C_C between droughted and well-watered plants for all experiments was statistically less than zero, P <0.01), demonstrating that photosynthesis is generally more limited by g_m in water-stressed plants.

Lower C_i-C_C in stressed plants suggested that the reduction in g_m was quantitatively more important than the reduction in A: the relative change in C_i–C_C in Fig. 4 can be calculated from modifications in A and g_m as $1–A(\text{low})/A(\text{control})\times g_m(\text{control})/g_m(\text{low})$. Depending on foliage and plant longevity, plants in drought-prone environments either avoid water stress by being functionally active in periods with ample soil water availability (ephemerals, drought-deciduous species) or sustain foliage during drought (tolerant species). As high turgor pressures are needed for foliage expansion, foliage growth is generally constrained to periods with relatively high water availability, and thus, even in drought-tolerant species, young fully-expanded leaves are not necessarily structurally acclimated to the harshest drought periods during the growing season. However, profound structural foliage modifications such as cell wall thickening and enhanced cell wall lignification can occur during drought stress periods (Escudero *et al.*, 1992; Sobrado, 1992; Henry *et al.*, 2000). Such changes are reflected in gradual increases in M_A, leaf density, and dry to fresh mass ratio during the drought (Kalapos, 1994; Kalapos *et al.*, 1996; Groom and Lamont, 1997; Nogués and Baker, 2000). After the drought cycle(s) such functional modifications are often maintained, indicating structural acclimation of foliage to drought stress (Rascio *et al.*, 1990; Kalapos, 1994; Kalapos *et al.*, 1996). This is supported by our meta-analysis, which showed that leaves became more robust during the drought (larger M_A in stressed plants; Fig. 4). While most studies have not monitored the recovery of g_m after drought stress, the study of Monti *et al.* (2006) demonstrated that g_m was reduced after drought stress, consistent with the drought-dependent structural foliage modifications. In fact, many studies have demonstrated that, although stomatal conductance may recover to pre-stress values, foliage photosynthesis rate rarely reaches the pre-stress value (Tenhunen *et al.*, 1987; Beyschlag *et al.*, 1990; Flexas *et al.*, 2001), suggesting either a decline in foliage photosynthetic potentials or in g_m. Recent studies have highlighted an important role of g_m in limiting photosynthesis recovery after water stress, but the effects seem to be variable, depending on species and specific environmental conditions (Galmés *et al.*, 2007*c*; Flexas *et al.*, 2009; Gallé *et al.*, 2009). Additional experimental work is needed to pinpoint the structural and physiological causes of reduced photosynthetic rates in foliage recovered from drought. We suggest that the functional implication of altered structure that is maintained after drought cycles is rigidification of the cell walls that is reflected in increased leaf bulk elastic modulus (ε), defined as the hydrostatic pressure change per unit change in leaf volume (change in relative leaf water content); larger ε after drought-stress is frequently observed (Bowman and Roberts, 1985; Abrams *et al.*, 1990; Kloeppel *et al.*, 1994), but not always (Fan *et al.*, 1994). Leaves with a larger ε lose less water for a given change in leaf water potential than leaves with a smaller ε; accordingly, leaves with more rigid cell walls can extract water from drier soils with given leaf water loss (Niinemets, 2001). While such modifications favourably affect leaf water relations, they inevitably result in a longer effective liquid-phase diffusion pathway in the foliage, possibly because of inherent increases in cell wall thickness and lower porosity of cell walls with increasing ε.

Moderate salt stress and water stress have broadly similar effects on g_m. Salt stress has been shown to decrease g_m in species as diverse as *Spinacia oleracea* (Delfine *et al.*, 1998), *Olea europaea* (Bongi and Loreto, 1989; Loreto *et al.*, 2003), and mangroves (Sobrado and Ball, 1999; Parida *et al.*, 2004), and the salt-stressed plants fitted the general patterns depicted in Fig. 4. In most cases, reductions in g_m and g_s explain salt-induced reductions in photosynthesis (Centritto *et al.*, 2003; Flexas *et al.*, 2004), but photosynthetic capacity is also reduced under chronic stress (Bongi and Loreto, 1989). As with prolonged water-stress and water-stress during plant development, enhanced growing medium salinity leads to profound modifications in leaf anatomy such as thickened cell walls, and smaller and more densely packed leaf cells (Bongi and Loreto, 1989; Qiu *et al.*, 2007), possibly explaining the long-term reduction in g_m in salt-stressed plants.

Acclimation to light gradients

Plant canopies commonly support large leaf area indices (leaf area per unit ground area), more than 4–5 $m^2\ m^{-2}$ in closed canopy ecosystems and even more than 10 $m^2\ m^{-2}$ in dense forests. Such large leaf area indices result in self-shading and strong light gradients within the plant canopies. Acclimation to light gradients during foliage formation is associated with strong, often 2–4-fold, increases in M_A, mainly because of increased leaf thickness (see Niinemets, 2007, for a review). A_{mass} varies little along light gradients (Ellsworth and Reich, 1993; Evans and Poorter, 2001; Fig. 4; Niinemets and Sack, 2006), suggesting that photosynthetic potentials of average leaf cells stay essentially constant along the light gradients. Although increases in leaf thickness are expected to result in a stronger reduction of light within the leaves (Terashima and Hikosaka, 1995), higher incident light and anatomical adaptations improving light penetration into the leaf interior, such as long palisade cells (Smith *et al.*, 1997), apparently compensate for potentially more extensive light gradients in thicker leaves. As the result of the positive scaling of M_A with light, A/area ($M_A A$/mass) increases strongly, 2–4-fold, with increasing light availability, reflecting stacking of foliage mesophyll per unit area. In high-light acclimated leaves with greater M_A and larger numbers of mesophyll cell layers, S_C/S is also larger, explaining the positive scaling of g_m/area with light (Terashima *et al.*, 2006). CO_2 drawdown, C_i–C_C, generally varies much less across the light gradients (Piel *et al.*, 2002; Warren *et al.*, 2003, 2007), suggesting similar limitations of photosynthesis due to g_m in leaves acclimated to different light availabilities (see Fig. 5 for an analysis of light effects on diffusion conductance and CO_2 drawdown in eight species). This constancy of CO_2 drawdown is accompanied by less variable g_m/mass than g_m/area (Fig. 5), further suggesting that light-dependent changes in g_m/area mainly reflect enhanced mesophyll and chloroplast surface area per unit total leaf area rather than altered foliage mesophyll diffusion characteristics.

The differences between light effects on area- and mass-based photosynthetic characteristics may sometimes be not that clear-cut. In fact, several environmental characteristics can vary with light in plant canopies. For instance, day-time air temperature is larger and humidity lower in the upper canopy. Average wind speeds also increase with increasing height in the canopy (see Niinemets and Anten, 2009, for a review). These factors can result in higher vapour pressure deficit between the leaves and atmosphere, and enhanced transpiration rate. In addition, water potentials become increasingly lower with increasing height in the canopy reflecting greater transpiration rates and counter balancing of the gravitational component of water potential. As the result of greater potential transpiration rates and lower inherent water potentials in the upper canopy, the leaves may sustain greater water stress in the upper canopy (Aasamaa *et al.*, 2004) and may develop structural acclimation responses compatible with drought adaptation, including greater foliage density and larger lignin concentrations (Niinemets and Kull, 1998). As noted above, such changes in response to increased water limitations can result in enhanced mesophyll diffusion limitations. Although g_m/area increased with increasing light availability in the canopy of Mediterranean sclerophyll *Quercus ilex* canopy, g_m/mass was negatively associated with light availability and this negative relationship was also reflected in higher C_i–C_C in higher light (Niinemets *et al.*, 2006). In fact, a meta-analysis of light effects in eight species suggests that g_m/mass and C_i-C_C are slightly lower in leaves grown in high light than in low-light (Fig. 5).

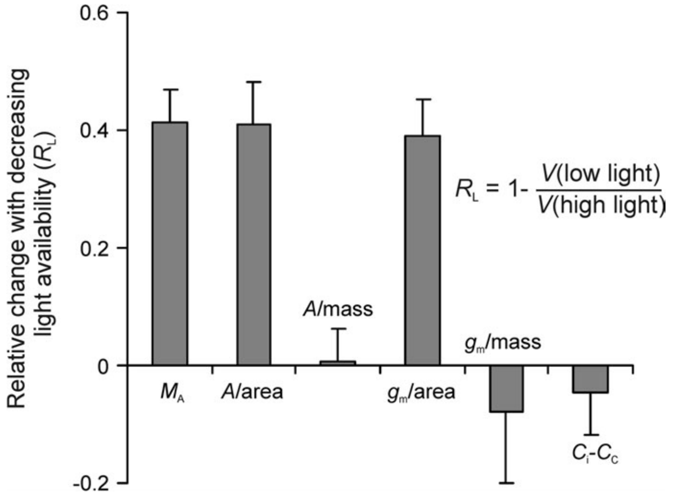

Fig. 5. Light-dependent modifications in leaf dry mass per unit area (M_A), leaf photosynthetic capacity per unit area and per unit mass and mesophyll diffusion conductance per unit area and per unit mass and corresponding CO_2 drawdown from substomatal cavities to chloroplasts. Relative light-dependent change (R_L) in each of these characteristics was calculated as shown in the figure (V denotes a given trait). The averages ±SE are based on eight tree and shrub species: *Acer mono*, *A. palmatum*, *A. rufinerve* (Hanba *et al.*, 2002), *Citrus paradisi*, *Prunus persica* (Lloyd *et al.*, 1992; Syvertsen *et al.*, 1995), *Juglans nigra*×*J. regia* (Piel *et al.*, 2002), *Fagus sylvatica* (Warren *et al.*, 2007), and *Quercus ilex* (Niinemets *et al.*, 2006).

Temperature effects and altitudinal variations

Temperatures during foliage growth and development strongly vary during the season and between the seasons. In addition, there are strong latitudinal and altitudinal temperature gradients. However, the direct effects of growth temperature on g_m have been studied in only few cases (Yamori *et al.*, 2006; Warren, 2008*a*). Leaf growth studies demonstrate that plants grown under lower temperatures have smaller leaves with smaller and more tightly packed cells (Woodward *et al.*, 1986; Tardieu *et al.*, 1999). This results in greater M_A due both to enhanced leaf density and thickness (Tardieu and Granier, 2000). Warmer temperatures, in contrast, are associated with greater leaf expansion and lower leaf thickness, provided higher temperatures are not interacting with greater water limitations. For instance, year-to-year variations in temperature during bud-burst and leaf development are known to alter M_A and foliage photosynthetic potentials with cool spring temperatures resulting in slower leaf expansion growth, and smaller and thicker leaves with larger M_A (Bassow and Bazzaz, 1998; Koike *et al.*, 2004). Such modifications are expected to result in stronger mesophyll diffusion limitations in leaves grown under lower temperatures. Consistent with these expectations, a study with *Spinacia oleracea* demonstrated greater CO_2 drawdown from substomatal cavities to chloroplasts (C_i–C_C) and greater limitation of photosynthesis by mesophyll diffusion conductance in plants grown at day-time temperatures of 15 °C relative to the plants grown at 30 °C (Yamori *et al.*, 2006). Similarly, preliminary studies in *Brassica oleracea* grown at 5 °C or 20 °C confirmed this trend with even more marked differences, with plants growing at 5 °C showing extremely low g_m at any temperature (Flexas *et al.*, 2008). However, a similar study with seedlings of the evergreen tree species *Eucalyptus regnans* did not find any effect of growth at 15 °C versus 30 °C on g_m or photosynthesis (Warren, 2008*a*).

Analogous responses have been found in response to altitudinal temperature gradients. M_A of temperate species increases from lower to higher altitudes, and this increase is associated with more densely packed cells with thicker cell walls at higher altitudes (Woodward, 1986; Körner and Woodward, 1987). Such anatomical modifications are expected to enhance mesophyll diffusion limitations of photosynthesis. The data of Kogami *et al.* (2001) demonstrate lower g_m/area and g_m/mass values in the temperate species *Polygonum cuspidatum* at higher altitudes and correspondingly larger drawdown of CO_2 (recalculated from Kogami *et al.*, 2001), indicating stronger mesophyll diffusion limitations. Analogously, Hawaiian *Metrosideros polymorpha* populations at higher elevations had greater M_A, and were predicted to have lower mesophyll diffusion conductance and greater CO_2 drawdowns than the populations at lower elevations (inferred by Evans and Loreto, 2000) (from whole-leaf carbon isotope discrimination data of Vitousek *et al.*, 1990). By contrast, a recalculation of the data of Shi *et al.* (2006) highlighted a reverse pattern, i.e. somewhat greater g_m and lower C_i-C_C in *Buddleja davidii* plants at 2400 m elevation than at 1300 m elevation. Of course, several environmental factors change with altitude, and, obviously, predicting the altitudinal trends in g_m on the basis of temperature only is far too simplistic.

Influence of nutrient limitations

Positive relationships of foliage photosynthetic potentials with foliage N and P contents are well known (Terry and Ulrich, 1973; Field and Mooney, 1986), and many models of canopy and ecosystem gas exchange are driven by relationships of photosynthesis with N (de Pury and Farquhar, 1997). The underlying biochemical response of photosynthesis to nutrient supply may be confounded by the response of g_m to nutrient availability. If, for example, elevated nutrient supply increases photosynthetic capacity, but g_m is unaffected, C_i–C_C will increase and cause greater relative diffusion limitation of photosynthesis.

Because of a series of modifications in leaf morphology and biochemistry with nutrient availability, it is not immediately evident how nutrient supply and/or leaf nutrient concentrations alter photosynthetic diffusion limitation. Based on the positive relationship of g_m with photosynthetic capacity, we expect g_m to increase with nutrient supply as long as the exposed chloroplast to leaf area ratio is positively affected by nutrient supply. An enhanced number of chloroplasts has been observed in response to an improved supply of a variety of nutrients (Spiller and Terry, 1980; Terashima and Evans, 1988; Laza *et al.*, 1993; Henriques, 2004), positively affecting g_m. However, the size of chloroplasts as well as stacking of thylakoids (grana number per chloroplast) also increases with increasing nutrient availability (Terashima and Evans, 1988; Laza *et al.*, 1993; Doncheva *et al.*, 2001), extending the liquid- and lipid-phase diffusion pathway in chloroplasts, and thereby negatively affecting g_m (Terashima and Evans, 1988). Apart from chloroplasts, enhanced nutrient supply is associated with lower M_A (see Poorter *et al.*, 2009, for a review), and larger cell size (Roggatz *et al.*, 1999) and reduced tissue density and cell wall lignification (Waring *et al.*, 1985; Niinemets *et al.*, 2001), positively affecting g_m at a given foliage photosynthetic capacity. So far, the empirical evidence for the effects of nutrient supply on g_m and C_i–C_C is equivocal. A reduction in g_m in response to low N supply has been found in some cases as for the herb *Phaseolus vulgaris* (von Caemmerer and Evans, 1991) and for seedlings of *Eucalyptus globulus* (Warren, 2004), but not always, as for the annual grass *Triticum aestivum* (von Caemmerer and Evans, 1991). In cases of positive nutrient availability effects on g_m, N supply affected photosynthetic capacity more strongly, such that the drawdown actually decreased somewhat under low N supply: average C_i–C_C=87 μmol mol^{-1} at high versus 78 μmol mol^{-1} at low N supply in *E. globulus* (Warren, 2004) and average C_i–C_C=81 μmol mol^{-1} at high versus 26 μmol mol^{-1} at low N supply in *P. vulgaris* (von Caemmerer and Evans, 1991). By contrast, no clear-cut differences were found in *T. aestivum* where g_m was unresponsive to nutrients (von Caemmerer and Evans, 1991). Leaf structural information is only available for *E. globulus*, and in this study, N-limitation increased M_A only by 10% (Warren, 2004), which is moderate compared with several-fold modifications in M_A under extreme nutrient limitations in some species (Gulmon and Chu, 1981). Available evidence suggests that nutrients do affect g_m, but the overall effect of these changes depends on both the effects of nutrients on g_m and on photosynthesis. While the existing data suggest a moderate effect of nutrients on diffusion limitations, for a more conclusive study of nutrient effects, experiments are needed using a larger range in nutrient supply on species with larger responses of leaf morphology and photosynthesis.

Interactions between environmental and internal variables and their effects on g_m

The effects of environmental variables on g_m have often been examined in isolation of other environmental drivers. However, as discussed in the section on acclimation to light gradients, several environmental variables often interact and simultaneously affect g_m. Such interactive effects on photosynthesis cannot generally be predicted on the basis of single factors (Valladares and Pearcy, 1997; Valladares *et al.*, 2008). Hence, understanding interactions is crucial to predict natural variation in g_m and photosynthesis. For instance, the response of g_m to changes in instantaneous temperature depends on previous temperature acclimation during growth in *Spinacia oleracea* (Yamori *et al.*, 2006) and in *Brassica oleracea* (Flexas *et al.*, 2008). Diaz-Espejo *et al.* (2007) in *Olea europaea* trees and Araujo *et al.* (2008) in *Coffea arabica* showed that the values of g_m of leaves exposed to similar daily-integrated radiation but at different positions within the canopy were different. This suggests that g_m at any given growth irradiance can be affected by other interacting factors such as temperature and leaf water availability. An interaction between light environment and age status of generative organs (sink–source relations) affected g_m values in the tropical fruit tree *Mangifera indica* (Urban *et al.*, 2008). Among the results shown at this Workshop, significant interactions affecting the response of g_m were demonstrated between water stress and the prevailing light conditions (Flexas *et al.*, 2009; Gallé *et al.*, 2009), among water stress, vapour pressure deficit, and species (Pérez-Martin *et al.*, 2009), and among light intensity, leaf position, and developmental stage (Monti *et al.*, 2009).

Genetic factors can interact with environmental drivers to determine g_m. In particular, the responses of g_m to a given environmental factor can differ among the genotypes of a single species. For example, the response of g_m to increased atmospheric ozone concentrations depends on the cultivar of *Phaseolus vulgaris* (Flowers *et al.*, 2007), and the response of photosynthesis and g_m to salinity stress differs among *Olea europaea* varieties (Centritto *et al.*, 2003). Different provenances of *Castanea sativa* (Lauteri *et al.*, 1997) had widely varying structural characteristics, photosynthetic potentials, and g_m values, further underscoring the importance of genetic variation.

Age-dependent alterations in g_m

Modifications in g_m *in leaves of different age*

Leaf development and senescence comprise a significant part of a leaf life-time, especially in some species with delayed foliage development (Miyazawa *et al.*, 2003). Accordingly, understanding the photosynthetic modifications during these periods is important to simulate leaf lifetime carbon gain. In developing leaves, cell number and size continuously increase and leaves become increasingly structured, including the formation of intercellular air space and distinct mesophyll layers. These structural modifications are accompanied by the multiplication of chloroplasts in each cell and the synthesis of chlorophyll and photosynthetic proteins, resulting collectively in age-dependent increases in photosynthesis (Tichá, 1985; Gratani and Ghia, 2002). As very young leaves essentially lack intercellular air-space, the values of g_m are initially very low (Miyazawa and Terashima, 2001; Eichelmann *et al.*, 2004), and gradually increase as the liquid-phase diffusion pathway length decreases and S_C/S increases due to increases in chloroplast number and number of mesophyll cells (Miyazawa *et al.*, 1998, 2003; Miyazawa and Terashima, 2001). After initial rapid development, foliage photosynthetic potentials and g_m reach the maximum values in fully-expanded young mature leaves (Hanba *et al.*, 2001; Eichelmann *et al.*, 2004).

After reaching the maximum values, foliage photosynthetic potentials are relatively constant or decrease at a slow rate (Wilson *et al.*, 2000, 2001; Niinemets *et al.*, 2004) that can speed up during environmental stress such as drought (Clifton *et al.*, 2002). This reduction in photosynthetic potentials is commonly associated with slow remobilization of limiting elements such as nitrogen (Wendler and Millard, 1996; Niinemets *et al.*, 2004). In addition, reduction in mass-based photosynthetic potentials can be associated with the accumulation of cell wall material, especially in leaves experiencing drought (e.g. larger M_A after long-term drought; Damour *et al.*, 2008). Both the reduction due to nutrient remobilization and cell wall accumulation can be accompanied by a reduction in g_m (Miyazawa and Terashima, 2001). As long as the reduction in g_m due to slow dismantling of photosynthetic machinery goes in parallel with reductions in photosynthetic potentials, the CO_2 drawdown should not necessarily change. By contrast, the accumulation of cell wall material and reduced porosity result in increased liquid-phase pathway length, and if this occurs without changes in photosynthetic potentials or such modifications are relatively small, cell wall modifications can increase the CO_2 drawdown. Although the detailed anatomical controls have not been studied so far, increasingly larger drawdowns in older non-senescent leaves have been observed (Grassi and Magnani, 2005).

The reduction of foliage photosynthetic rates speeds up after the onset of leaf senescence (Rosenthal and Camm, 1997; Niinemets *et al.*, 2004). This is accompanied by a strong reduction in g_m (Loreto *et al.*, 1994; Evans and Vellen, 1996; Delfine *et al.*, 1999; Flexas *et al.*, 2007*b*; Zhang *et al.*, 2008). In one study conducted in wheat (*Triticum*), the drawdown from substomatal cavities to chloroplasts was essentially invariable (Evans and Vellen, 1996), while the drawdown increased with increasing leaf age in another study in wheat (Loreto *et al.*, 1994) and also in studies with herbs like *Cypripedium flavum* (Zhang *et al.*, 2008), *Spinacia* *oleracea* (Delfine *et al.*, 1999), and *Arabidopsis thaliana* (Flexas *et al.*, 2007*b*). Reduction of both g_m and A is expected as senescence is associated with the dismantling of the photosynthetic apparatus, i.e. reduced S_C/S and content of photosynthetic proteins (Evans and Vellen, 1996). The extent to which mesophyll diffusion limitations change during senescence obviously depends on age-dependent changes in cell-wall properties and internal architecture, on the degree of reduction of chloroplast number and size, modifications in the positioning of chloroplasts along the cell walls as well as on changes in average content of photosynthetic enzymes in functional chloroplasts, and may also depend on modifications in aquaporin conductance. While the early steps of leaf ageing can be associated with cell wall accumulation and lignification, gradual degradation and distortion of cell walls has been observed in senescing leaves (Lee and Chen, 2002; Nemoto *et al.*, 2004). Stress-dependent accelerated senescence has been found to result in local thickening of cell walls due to pectic intrusions (Günthardt-Goerg and Vollenweider, 2007). Such profound modifications in cell wall structure can importantly alter liquid-phase diffusion conductance. Obviously, more experimental work is needed for quantitative assessment of the structural, physiological, and biochemical controls on g_m and photosynthesis in senescing leaves.

Evergreen leaves constitute a special case of age-dependent modifications in leaf structure and functioning. Evergreen leaves are exposed to a variety of environmental stresses during their life-span, and this is associated with large increases in M_A, leaf density, and dry to fresh mass ratio with increasing leaf age (Niinemets and Lukjanova, 2003; Niinemets *et al.*, 2005). There is a gradual decline in nitrogen contents per dry mass, but nitrogen contents per area decline with slower rate or remain essentially constant (Escudero *et al.*, 1992; Niinemets *et al.*, 2005), except for a relatively short period of intensive N and P retranslocation to growing shoots in spring, followed by refilling of leaf N pools (Cherbuy *et al.*, 2001). These differences between area- and mass-based trends in nutrient contents indicate that modifications in M_A primarily reflect the accumulation of cell wall material. These chemical and structural changes are associated with concomitant reductions in foliage photosynthetic potentials (Niinemets *et al.*, 2005; Ethier *et al.*, 2006) (Fig. 6b). Part of this reduction is due to shading of older foliage by new foliage and the reallocation of foliar nitrogen from proteins limiting photosynthesis at light-saturation such as Rubisco to pigment-binding light-harvesting proteins (Niinemets *et al.*, 2006). Photosynthetic reductions can also result from the association of a greater fraction of N with the cell walls (see Takashima *et al.*, 2004, for an analysis of nitrogen distribution between cell walls and cytosol in leaves with contrasting structure).

At any rate, the accumulation of cell wall material is expected to lead to reduced liquid-phase diffusion conductance and enhanced CO_2 drawdown (Evans *et al.*, 2009). Such reduced g_m values (Fig. 6a) and greater CO_2 drawdowns (Fig. 6c) have been observed in leaves of several Mediterranean evergreen sclerophylls (Niinemets *et al.*, 2005). On the other hand, Ethier *et al.* (2006) observed that the reduction in foliage photosynthetic potentials was quantitatively as important as the reduction in g_m such that CO_2 drawdown did not depend on leaf age. However, these

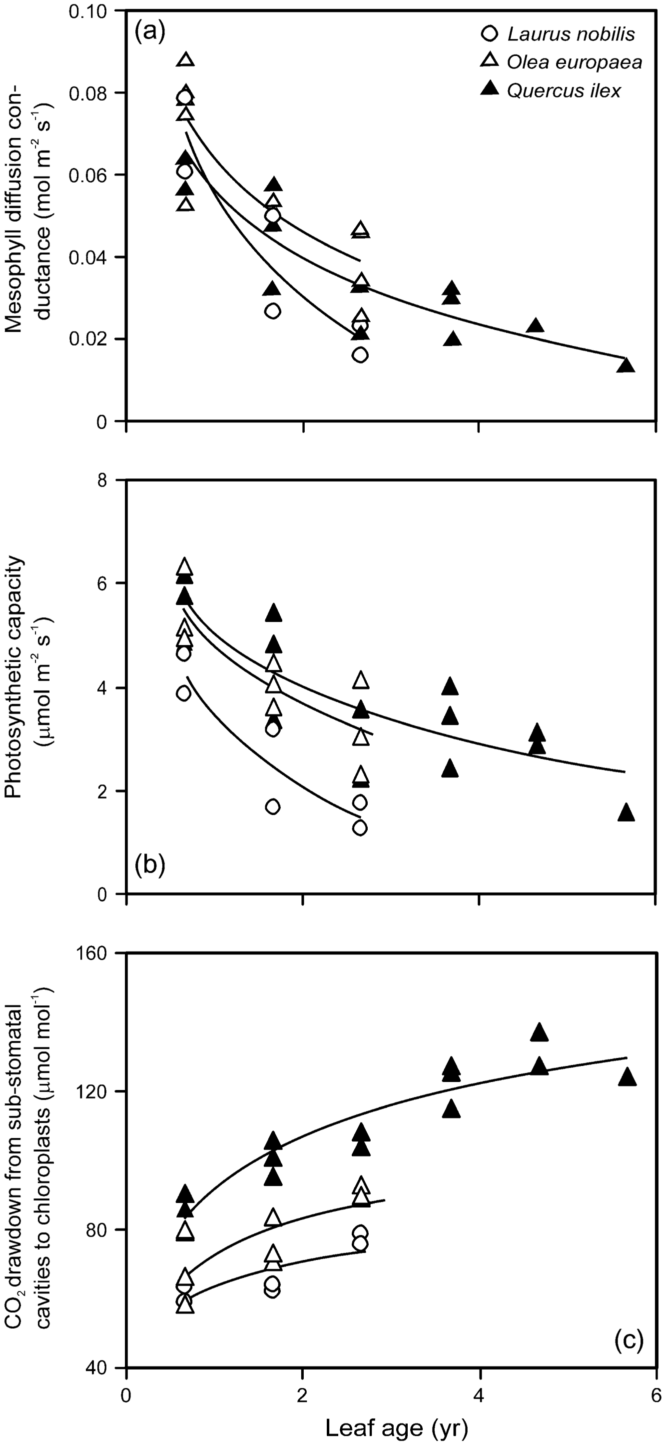

Fig. 6. Age-dependent modifications in mesophyll diffusion conductance (a), photosynthetic capacity (b) and in CO_2 drawdown from substomatal cavities to chloroplasts (c) in three Mediterranean evergreen sclerophyllous species (modified from Niinemets *et al.*, 2005) estimated at average ambient CO_2 concentration 335 μmol mol^{-1}. Data were fitted by non-linear regressions in the form of $y=ax^b$ (all significant at P <0.01).

discrepancies may reflect the extent to which the ageing foliage becomes shaded and re-acclimated to altered light conditions as such re-acclimation may change A more than g_m. In a study with Mediterranean species, age-dependent re-acclimation of photosynthetic machinery to shade reduced CO_2 drawdown (Niinemets *et al.*, 2006), but the effect of modified g_m was quantitatively more important than the effect of re-acclimation (Fig. 6). Depending on foliage longevity, current-year leaves may comprise only a small fraction of total canopy foliage, and thus understanding the functioning of older foliage is of key significance in predicting the canopy carbon gain in evergreens.

Despite the functional group differences in duration of and physiological and structural modifications during senescence and ageing, there is evidence for increased CO_2 drawdown (i.e. increased photosynthesis limitation by g_m) in older leaves in species as diverse as Mediterranean evergreen sclerophyll trees and shrubs (Niinemets *et al.*, 2005), deciduous temperate trees (Grassi and Magnani, 2005), the annual herbs *Arabidopsis thaliana* (Flexas *et al.*, 2007*b*) and *Spinacia oleracea* (Delfine *et al.*, 1999), annual grass *Triticum durum* (Loreto *et al.*, 1994) or the alpine orchid *Cypripedium flavum* (Zhang *et al.*, 2008).

Changes in g_m *in plants of different age*

Foliage photosynthesis rates decrease with increasing tree age and size (Yoder *et al.*, 1994; Niinemets, 1997; Bond, 2000). This reduction was initially related to reductions in foliage stomatal conductance due to longer water transport pathways and accordingly to a greater drop of leaf water potential (Yoder *et al.*, 1994). However, possibly because of greater water stress in leaves of large trees, M_A also increases with increasing plant age and size (England and Attiwill, 2006; Niinemets, 2006; He *et al.*, 2008). In conifers, this increase is primarily associated with enhanced foliage density (Niinemets, 1997), while both thickness and density increase in broad-leaved species (Niinemets *et al.*, 1999; He *et al.*, 2008). Age-dependent increases in M_A are associated with concomitant reductions in mass-based foliage photosynthetic rates (Niinemets, 2002). As foliage N contents do not necessarily change with increasing tree size and age (Niinemets, 2002), it is tempting to speculate that the age-dependent decrease in photosynthetic rates in older trees is at least partly driven by greater mesophyll diffusion limitations. Recent work demonstrates that g_m is lower in taller trees (Fig. 7; Woodruff *et al.*, 2008). Yet, there was no general size-dependence of CO_2 drawdown, albeit the drawdown in medium-sized trees (34 m) was larger than in smaller trees (Fig. 7, inset). Preliminary results in Mediterranean sclerophylls suggest that g_m is smaller and CO_2 drawdown larger in older and bigger seedlings as compared with one-year-old seedlings (J Flexas, unpublished results). Thus, variation in g_m may constrain photosynthesis to a differing degree in trees of different size, and deserves to be added to the list of factors potentially causing photosynthetic decline in older trees.

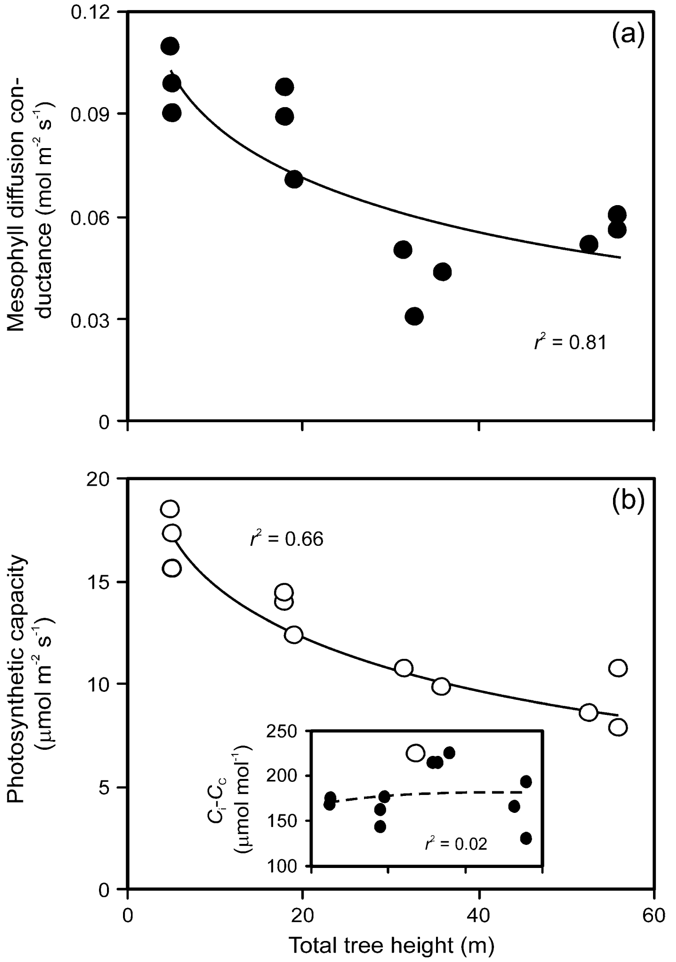

Fig. 7. Variation in mesophyll diffusion conductance (a), leaf photosynthetic capacity (b) and in CO_2 drawdown from substomatal cavities (C_i) to chloroplasts (C_C) (inset in b) with total tree height in the temperate evergreen conifer *Pseudotsuga menziesii* (modified from Woodruff *et al.*, 2008) measured at an ambient CO_2 concentration of 385 μmol mol^{-1}. The measurements were conducted in four different stands with contrasting total height (mean sampling heights 5.0, 18.3, 33.5, and 55.0 m). Data fitting as in Fig. 6. The regressions in the main panels were significant at P <0.001, the regression in the inset was non-significant (P >0.5).

What consequences do different g_m values have for photosynthesis in the field?

In the previous sections, it has been shown how g_m varies in dependence on foliage structure and leaf photosynthetic potentials, in response to environmental stresses of various duration and in leaves and in plants of different age. As the drawdown of CO_2 from substomatal cavities to chloroplasts often varies in leaves of differing structural and physiological characteristics and in response to various environmental and ontogenetic stimuli, it is pertinent to ask to what extent such differences in the CO_2 drawdown due to g_m modify foliage photosynthesis in field environments. Because of the strong dependence of photosynthesis on CO_2 concentration at current relatively low ambient CO_2 concentrations, any reduction of CO_2 inside the chloroplasts is reducing the realized net assimilation rates at given biochemical investments in photosynthetic machinery. However, the sensitivity of foliage photosynthesis to any given value of g_m depends on the overall diffusion conductance from the ambient atmosphere (C_a) to the chloroplasts, i.e. it is also affected by boundary layer conductance (g_b) and by stomatal conductance (g_s). As in the field, leaves are generally well coupled to the atmosphere and the contribution of boundary layer conductance relative to g_s and g_m is small (except for large leaves under low wind speed; Stokes *et al.*, 2006), we consider only g_s and g_m in the current analysis, i.e. assume that the CO_2 concentrations outside and inside the boundary layer are equal. In a steasdy-state, net assimilation rate is equal to:

$$A = g_s(C_a - C_i) = g_m(C_i - C_C) \tag{3}$$

As the total diffusion conductance from the ambient air is the sum of the inverse serial conductances, we can express the net assimilation rate as the product of total conductance and the diffusion gradient from ambient air to chloroplasts:

$$A = \frac{1}{\frac{1}{g_s} + \frac{1}{g_m}}(C_a - C_c) \tag{4}$$

This equation demonstrates that the total diffusion conductance depends on both g_s and g_m, but also that for numerically widely differing g_m and g_s values, the overall conductance and the resulting effect on photosynthesis will be dominated by the smaller component. To simulate foliage net assimilation rates, a fully iterative approach was used with $A=f(C_C)$, $C_C=g(A, C_i, g_m)$, $C_i=h(g_s, C_a, C_C, g_m)$, where $f(x)$, $g(x)$, and $h(x)$ denote functions of given argument(s) x.

These simulations demonstrate that A increases with g_m (Fig. 8a) due to progressively lower drawdowns C_i–C_C (Fig. 8b) and C_a–C_C (Fig. 8c), but also that the increase of A with g_m is curvilinear with a maximum A reached at progressively higher values of g_m as g_s increases (Figs 8a, 9a). This ceiling on A is imposed by limited g_s that starts to dominate overall diffusion conductance at higher g_m (Fig. 9a). For the value of g_m at which dA/dg_m is minimal, a g_m/g_s ratio between 1.3-1.7 is obtained, in accordance with the literature. It is important to highlight that it would be difficult for the leaf to compensate for the decreases in g_s observed under natural conditions, for instance due to water stress, as the total conductance is the sum of conductances in series. A leaf with a g_s of 0.15 mol m^{-2} s^{-1} and g_m of 0.2 mol m^{-2} s^{-1} has a total conductance to CO_2 (g_{tCO_2}) of 0.086 mol m^{-2} s^{-1}. If stomata close and g_s is decreased to 0.1 mol m^{-2} s^{-1}, g_m would need to rise as high as to 0.6 mol m^{-2} s^{-1} to keep g_{tCO_2} constant, and maintain the same value of A. That high value cannot be achieved by most species, due to anatomical and structural limitations as previous sections have shown. Even for herbaceous plants with the highest g_m values, never a g_m/g_s ratio of 6 has been observed. The

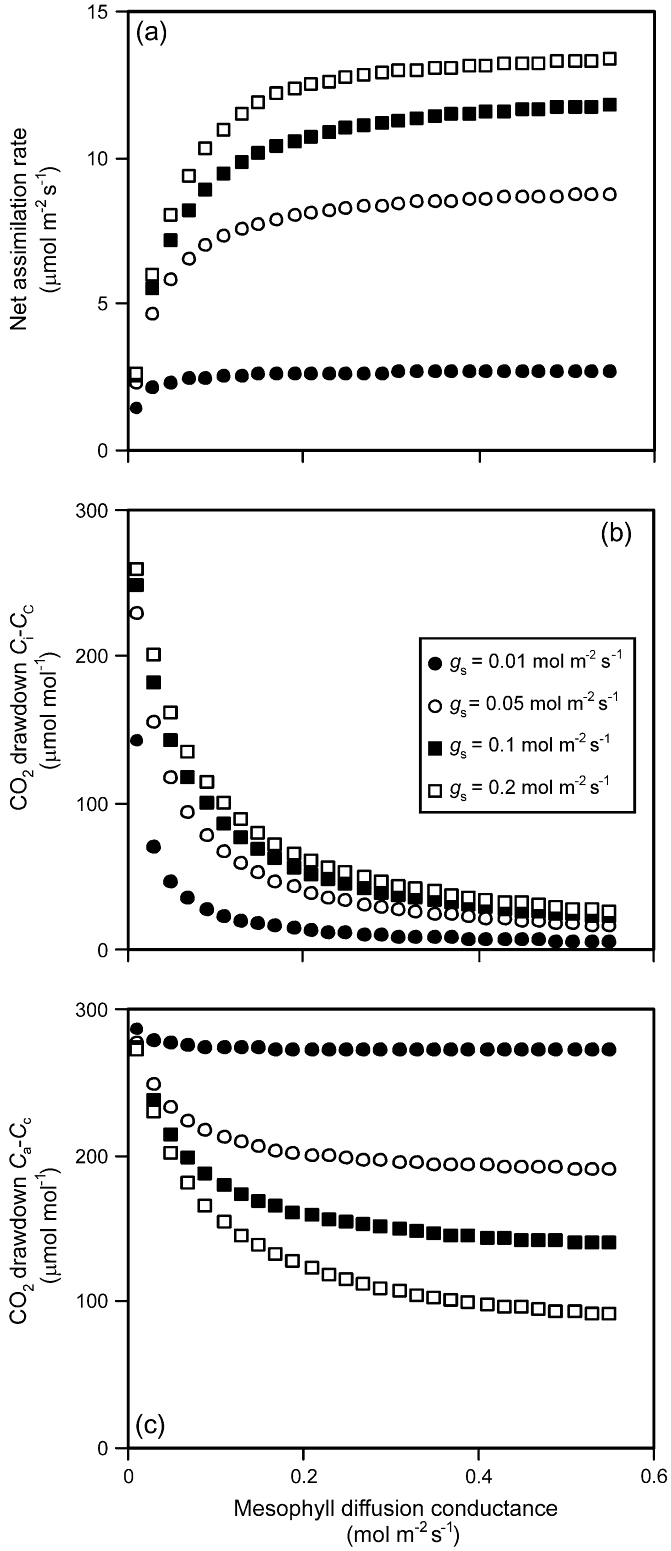

Fig. 8. Simulated dependencies of net assimilation rate (*A*) (a), CO_2 drawdown from substomatal cavities (C_i) to chloroplasts (C_C) (b) and CO_2 drawdown from ambient air (C_a) to chloroplasts (C_C) on mesophyll diffusion conductance in leaves with differing stomatal conductance (g_s). Foliage photosynthesis was simulated using the Farquhar *et al.* (1980) photosynthesis model for light-saturated conditions with a maximum carboxylase activity of Rubisco (V_{cmax}) of 50 μmol m^{-2} s^{-1}, the capacity for photosyn-

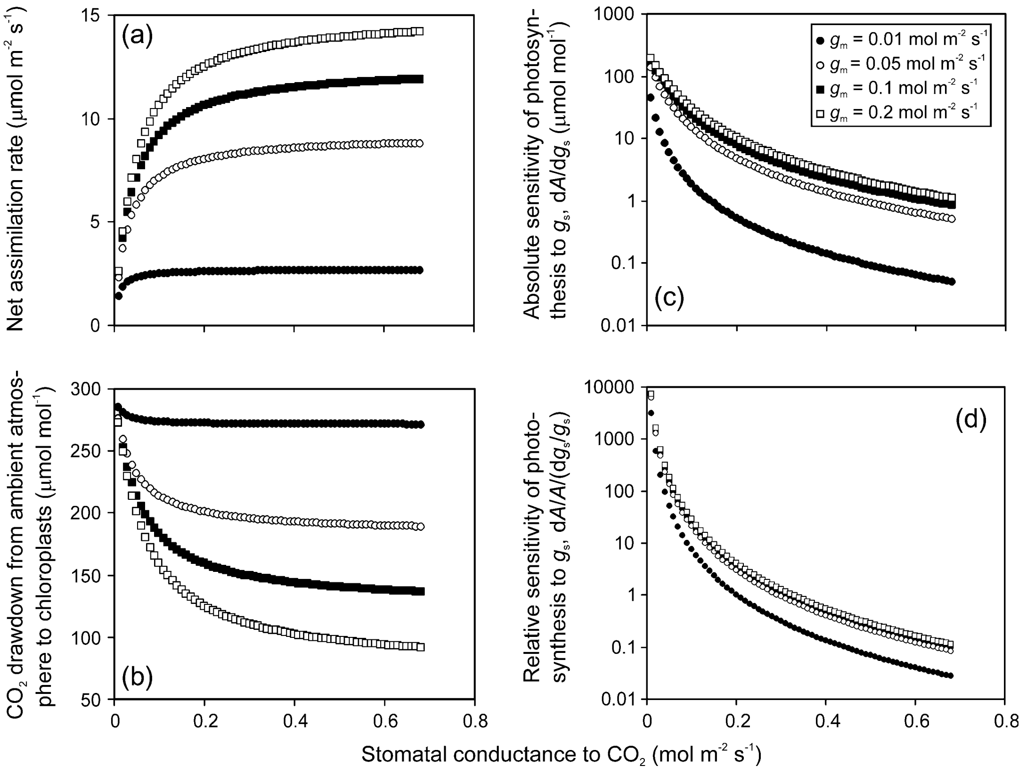

Fig. 9. Stomatal controls on A (a), CO$_2$ drawdown C_a–C_C (b) and absolute (c) and relative (d) sensitivity of A to changes in stomatal conductance for different values of mesophyll diffusion conductance. The absolute sensitivity is the change in A per unit change in g_s, while the relative sensitivity is the absolute sensitivity multiplied by g_s/A, correcting for differences among leaves with varying A, for instance due to differences in g_m (Fig. 8). The simulations were conducted as in Fig. 8 using the same parameters of Farquhar *et al.* (1980) model.

major implication of equation 4 is that photosynthesis is less sensitive to g_m when stomata are more closed (see Fig. 8 for simulations with different values of stomatal conductance and Fig. 9 for a sensitivity analysis). All these simulations were conducted at saturating light, where the drawdowns are the largest. Thus, these simulations provide the outer envelope of diffusional controls of photosynthesis.

The simulation analysis presented here suggests that the key effect of differences in g_m among leaves with varying structure is the redistribution of diffusion limitations between stomata and mesophyll. As discussed above, leaves with large M_A and low g_m are characteristic of stressful environments, in particular, environments with low water availability. Furthermore, adjustment to a variety of stresses commonly leads to reduced g_m and increased M_A. A common response to limited water availability is a drastic reduction of g_s to conserve water (see Flexas and Medrano, 2002, for a review). Our simulation analysis suggests that, under such highly stressful conditions, low g_m in structurally robust leaves is not necessarily exerting a major control over photosynthesis as the drawdown, C_a–C_C is primarily dominated by stomata (Fig. 8c). Conversely, in leaves with inherently low g_m, any reduction in stomatal conductance and thus, conservation of water use, affects photosynthesis less than in leaves with larger g_m (Fig. 9b–d). Such differences in the sensitivity of net assimilation to water conservation among leaves with varying g_m constitute a major factor functionally differentiating various plant functional types.

The situation can be radically different under non-stressed conditions when stomata are open. Considering leaves with given biochemical investments in photosynthetic machinery, under such conditions, inherently low g_m results in strongly reduced assimilation rates compared with leaves with a larger g_m (Figs 8a, 9a). Thus, reduced photosynthesis, i.e. inherently high CO$_2$ drawdown (Fig. 3) under non-stressed conditions due to low g_m is the cost that structurally robust leaves pay for their capacity to tolerate stressful conditions.

Conclusions

Strong co-ordination of g_m and foliage photosynthetic potentials has been suggested. However, there is increasing

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thetic electron transport (J_{max}) of 2.5 V_{cmax} and the mitochondrial respiration rate of R_d=0.05V_{cmax}. Rubisco kinetic characteristics were those from Bernacchi *et al.* (2001). Ambient CO$_2$ concentration was set at 370 μmol mol^{-1} and leaf temperature at 25 °C. A complete circular iteration was used where A is a function of C_C, V_{cmax}, J_{max}, R_d; C_C is a function of C_i, A, g_m; C_i is a function of C_C, g_s, C_a.

evidence that the correlations between g_m and A are variable, and CO_2 drawdown from substomatal cavities to chloroplasts can vary largely. Analogously, there are strong correlations between A and stomatal conductance (g_s), but these correlations also strongly differ, and the ratio A/g_s, the intrinsic water use efficiency, equivalent to CO_2 drawdown from the ambient atmosphere to substomatal cavities, varies largely among plants. There is no reason to expect the mesophyll CO_2 diffusion conductance to be more conserved than the intrinsic plant water use efficiency. Stress-dependent and age-dependent alterations in g_m further result in significant changes in the limitation of photosynthesis by mesophyll diffusion. Consideration of such effects is of key significance in simulating plant photosynthesis in the field.

The major implication of altered g_m is the re-distribution of overall diffusion limitation between stomata and mesophyll. With increasing the degree of mesophyll diffusion limitations, plant photosynthesis becomes less sensitive to fluctuations in stomatal conductance. While this results in less variable photosynthetic rates during the stress periods when stomatal conductance is low, inherently low g_m impairs plant photosynthetic productivity under favourable conditions.

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