

The role of mesophyll conductance in the economics of nitrogen and water use in photosynthesis

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Abstract A recent resurgence of interest in formal optimisation theory has begun to improve our understanding of how variations in stomatal conductance and photosynthetic capacity control the response of whole plant photosynthesis and growth to the environment. However, mesophyll conductance exhibits similar variation and has similar impact on photosynthesis as stomatal conductance; yet, the role of mesophyll conductance in the economics of photosynthetic resource use has not been thoroughly explored. In this article, we first briefly summarise the knowledge of how mesophyll conductance varies in relation to environmental factors that also affect stomatal conductance and photosynthetic capacity, and then we use a simple analytical approach to begin to explore how these important controls on photosynthesis should mutually co-vary in a plant canopy in the optimum. Our analysis predicts that when either stomatal or mesophyll conductance is limited by fundamental biophysical constraints in some areas of a

canopy, e.g. reduced stomatal conductance in upper canopy leaves due to reduced water potential, the other of the two conductances should increase in those leaves, while photosynthetic capacity should decrease. Our analysis also predicts that if mesophyll conductance depends on nitrogen investment in one or more proteins, then nitrogen investment should shift away from Rubisco and towards mesophyll conductance if hydraulic or other constraints cause chloroplastic CO_2 concentration to decline. Thorough exploration of these issues awaits better knowledge of whether and how mesophyll conductance is itself limited by nitrogen investment, and about how these determinants of photosynthetic CO_2 supply and demand co-vary among leaves in real plant canopies.

Keywords Mesophyll conductance · Internal conductance · Water use efficiency · Nitrogen use efficiency · Optimisation

Introduction

The efficiency with which plants use nitrogen and water in photosynthesis differs greatly among species, and over time and among leaves for a given species. These differences are important in determining the distribution, dispersal and survival of species, particularly in a rapidly changing climate, and recent decades have brought many advances in our understanding of the economics and physiology of photosynthetic nitrogen and water use. Mesophyll conductance (g_m) can have a large effect on photosynthetic nitrogen use efficiency (PNUE) and water use efficiency (WUE) (the ratios of leaf net CO_2 assimilation rate to leaf nitrogen content and transpiration rate, respectively) and on their relationship to one another.

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However, although a great deal of experimental work in the last decade has improved our knowledge of the magnitude of g_m , and how it is affected by environmental variables, much less emphasis has been placed on the role of g_m in photosynthetic resource economy. The objectives of this article are to review the role of mesophyll conductance in water and nitrogen economy, and to identify critical questions for future research. In this article, we define g_m as the diffusive conductance between the intercellular air spaces and the sites of carboxylation in chloroplasts (although it should be kept in mind that none of the methods currently used to measure g_m actually measures the diffusive conductance directly) (symbols are defined and units given in Table 1).

We will consider the role of g_m in resource economy from the perspective of two questions. First, how does g_m vary among leaves in a canopy in relation to the other major determinants of photosynthetic rate—stomatal conductance (g_s), photosynthetic capacity and environmental variables such as irradiance and CO_2 concentration? Second, what are the economic implications of these patterns of variation in g_m ? In particular, what patterns would we expect to see if these variables were optimally coordinated in such a way as to maximise photosynthesis, subject to finite supplies of transpirable water and photosynthetic nitrogen? The first question is addressed by a review of experimental physiology, while the second, by formal analysis of optimal resource allocation.

How does g_m vary in relation to g_s , photosynthetic capacity and environment?

The current understanding of g_m has been recently reviewed (Flexas et al. 2012), as have the mechanisms regulating g_m (Evans et al. 2009), and the ecophysiological and ecological significance of g_m been (Niinemets et al. 2009a, b; Terashima et al. 2011). These articles are a useful starting point for exploring the wider role of g_m in plant physiology. Given the recently realised role of g_m in economics of photosynthesis, below we briefly review those factors determining, and/or correlated with g_m that are of special importance for canopy-scale variation in the economics of photosynthesis. When evaluating the empirical data, an important caveat is that none of the methods currently used to measure g_m actually measure that diffusive conductance directly (Tholen et al. 2012), and all the methods are subject to various assumptions (e.g. Warren 2006). Hence, correlations of g_m with gas exchange parameters and dynamic responses of g_m could in some instances be artefacts of the measurement technique. For example, apparent light responses of g_m could be due to incorrect estimation of electron transport by chlorophyll fluorescence (Evans 2009), or failure to account correctly for the effect of PEPC carboxylation on isotope discrimination (Douthe et al. 2012). Nevertheless, stringent evaluation of the size of errors and uncertainties in g_m (Douthe et al. 2012; Douthe et al. 2011; Flexas et al. 2012) suggests

Table 1 Symbols referred to in the main text (symbols used only in the Appendix are defined there)

Definition	Symbol	Units
Net CO_2 assimilation rate (daily average)	A (A_d)	$\mu\text{mol m}^{-2} \text{s}^{-1}$
Ambient CO_2 mol fraction	c_a	$\mu\text{mol mol}^{-1}$
Chloroplastic CO_2 mol fraction	c_c	$\mu\text{mol mol}^{-1}$
Intercellular CO_2 mol fraction	c_i	$\mu\text{mol mol}^{-1}$
Mesophyll conductance per unit nitrogen	χ_m	$\mu\text{mol s}^{-1} \text{mmol}^{-1}$
Mesophyll conductance per unit Rubisco nitrogen	χ_{mv}	$\mu\text{mol s}^{-1} \text{mmol}^{-1}$
Carboxylation capacity per unit nitrogen	χ_v	$\mu\text{mol s}^{-1} \text{mmol}^{-1}$
Marginal carbon product of water (setpoint)	$\partial A/\partial E$ (λ^{-1})	$\mu\text{mol mmol}^{-1}$
Marginal carbon product of nitrogen (setpoint)	$\partial A_d/\partial N$ (v^{-1})	$\mu\text{mol s}^{-1} \text{mmol}^{-1}$
Leaf transpiration rate	E	$\text{mmol m}^{-2} \text{s}^{-1}$
Total conductance to CO_2	g	$\text{mol m}^{-2} \text{s}^{-1}$
Mesophyll conductance to CO_2	g_m	$\text{mol m}^{-2} \text{s}^{-1}$
Stomatal conductance to CO_2	g_s	$\text{mol m}^{-2} \text{s}^{-1}$
Ratio of mesophyll to stomatal conductance	γ	Unitless
Photorespiratory CO_2 compensation point	Γ_*	$\mu\text{mol mol}^{-1}$
Slope of CO_2 demand (A vs c_c) curve	k	$\text{mol m}^{-2} \text{s}^{-1}$
Effective Michaelis constant for RUBP carboxylation	K'	$\mu\text{mol mol}^{-1}$
Leaf photosynthetic nitrogen content	N	mmol m^{-2}
Leaf Rubisco nitrogen content	N_v	mmol m^{-2}
Photosynthetic nitrogen use efficiency	PNUE	$\mu\text{mol s}^{-1} \text{mmol}^{-1}$
Water use efficiency	WUE	$\mu\text{mol mmol}^{-1}$

Table 2 Summary of light-saturated rate of photosynthesis at ambient CO₂ (A_{\max}), mesophyll conductance (g_m) and the drawdown from sub-stomatal cavities to sites of carboxylation ($c_i - c_c$) in 50 C₃ species

	n (g_m) ¹	Species (g_m) ²	n (g_s) ³	Species (g_s) ⁴	A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	g_m ($\text{mol m}^{-2} \text{s}^{-1}$)	$c_a - c_i$ ($\mu\text{mol mol}^{-1}$)	$c_i - c_c$ ($\mu\text{mol mol}^{-1}$)	$c_a - c_c$ ($\mu\text{mol mol}^{-1}$)
Angiosperms										
Herbaceous dicot	27	9	16	8	18 (1) ^a	0.27 (0.04) ^a	0.29 (0.02) ^a	126 (13)	68 (6) ^a	197 (13)
Herbaceous monocot	20	3	9	2	24 (3) ^b	0.23 (0.03) ^{ab}	0.35 (0.04) ^b	111 (11)	77 (7) ^{ab}	189 (15)
Woody deciduous	77	17	28	13	11.2 (0.5) ^c	0.17 (0.02) ^{bc}	0.14 (0.01) ^c	136 (7)	88 (3) ^b	214 (7)
Woody evergreen	51	20	38	15	9.7 (0.6) ^c	0.21 (0.02) ^{ab}	0.11 (0.01) ^c	109 (9)	91 (4) ^c	202 (9)
Gymnosperms										
Evergreen	6	4	6	6	8.6 (0.9) ^c	0.09 (0.01) ^c	0.12 (0.03) ^c	162 (15)	91 (18) ^{abc}	253 (27)
Grand mean	181				13.1 (0.5)	0.20 (0.01)	0.178 (0.009)	123 (5)	85 (2)	207 (5)

Data are for fully developed, non-senescent leaves of plants that are neither salt nor water stressed. Measurements were made at ambient CO₂ concentrations (360–400 $\mu\text{mol mol}^{-1}$) between 20 and 30 °C. In cases where $c_i - c_c$ was not reported, it was calculated from published A and g_m : $c_i - c_c = A/g_m$. Input data were mean values for each species/treatment combination, rather than individual measurements. Data are means with one standard error in parentheses. Redrawn from (Warren 2008b)

Values with different letters were significantly different ($p > 0.05$, LSD post-hoc tests)

- 1 Number of reps for g_m , A_{\max} and $c_i - c_c$
- 2 Number of species for g_m , A_{\max} and $c_i - c_c$
- 3 Number of reps for g_s , $c_a - c_i$ and $c_a - c_c$
- 4 Number of species for g_s , $c_a - c_i$ and $c_a - c_c$

that at least some of the observed dynamic responses of g_m are real. Re-evaluation of published data is well beyond the scope of this article, and thus, for the remainder of this article, we assume that measured g_m is real.

g_m has been estimated for more than 100 species (Flexas et al. 2012), and thus, we have a reasonable grasp of trends among species and relationships of g_m with other photosynthetic parameters. For example, a review published in 2008 established that among 50 species, the mean drawdown from c_i to c_c (intercellular to chloroplastic CO₂ mol fraction) under saturating light at a c_a (ambient CO₂ mol fraction) of around 360 $\mu\text{mol mol}^{-1}$ was on the order of 85 $\mu\text{mol mol}^{-1}$ (Table 2) versus 123 $\mu\text{mol mol}^{-1}$ for the drawdown from c_a to c_i due to stomatal conductance. In terms of a limitation of photosynthesis, on average g_m accounts for around 40 % of the decrease in CO₂ concentration between the atmosphere and sites of carboxylation (Warren 2008b).

Anatomical traits, such as cell wall thickness and chloroplast distribution, are amongst the stronger determinants of mesophyll conductance. Most available evidence suggests that for most species, the bulk of the resistance to CO₂ movement is in the liquid phase rather than the gaseous phase (Parkhurst and Mott 1990), and the potential for CO₂ diffusion in the liquid phase is a function of cell wall thickness (Miyazawa and Terashima 2001; Nobel 1991; Scafaro et al. 2011; Terashima et al. 2011) and the surface area of mesophyll cells or chloroplasts exposed to the intercellular air spaces (Laisk et al. 1970; Nobel et al. 1975; Evans et al. 1994; Terashima et al. 2011).

A major influence on our understanding of what limits g_m has come from observations that g_m can change rapidly and independently of leaf anatomy (e.g. due to drought or leaf temperature), supporting views that g_m is also at least partially biochemical in nature. Carbonic anhydrases (CA) could play a role on the regulation of g_m by means of changing the nature of the diffusing molecule (i.e. HCO₃⁻ rather than or in addition to CO₂), although the effects of genetic modification of CA on g_m are small and inconsistent (Flexas et al. 2012). Aquaporins might also play a role in g_m by facilitating CO₂ diffusion through membranes (Cooper and Boron 1998; Nakhoul et al. 1998). Indeed, altered expression of aquaporins has been shown to result in changes in membrane permeability to CO₂ (Uehlein et al. 2008; Heckwolf et al. 2011).

Mesophyll conductance is regularly correlated with gas exchange parameters—in addition to, or perhaps because of, the underlying limitation of g_m by anatomical and biochemical factors. Among species, there is a positive relationship between rates of photosynthesis and g_m (Epron et al. 1995; Loreto et al. 1992; Harley et al. 1992; von Caemmerer and Evans 1991; Warren et al. 2003), but there is wide variation in this relationship and thus in $c_i - c_c$

(Warren and Adams 2006). In addition to the large variability in $c_i - c_c$ at any given g_m , there is a systematic trend with $c_i - c_c$ being larger for species with small g_m (and/or low A). When the relationship of g_m with A is broken down further, it is apparent that relationships vary among species (Hanba et al. 2001) and tend to be weaker within species. g_m also tends to be positively correlated with g_s , but as with $g_m - A$ correlations the relationship of g_m with g_s varies among species and among cultivars (Soolanayakanahally et al. 2009; Barbour et al. 2010) and may be weaker within species. A review by Flexas et al. (2008) suggested that g_s and g_m responded in parallel to irradiance, CO_2 , temperature and drought stress. However, under some conditions, changes in g_m and g_s are independent of each other. For example, water deficits may reduce g_s more than g_m and thereby increase the ratio g_m/g_s (Duan et al. 2009; Galmes et al. 2011); during midday depression of photosynthesis, an initial increase of g_m was accompanied by a simultaneous decrease of g_s (Pons and Welschen 2003). Partial independence of g_s and g_m has been observed with regard to their temperature response (Yamori et al. 2006; Warren and Dreyer 2006; Scafaro et al. 2011). Two recent studies reported that g_s was reduced by increases in vapour pressure deficit (Warren 2008a) and exogenous abscisic acid (ABA) (Vrabel et al. 2009), whereas neither vapour pressure deficit nor ABA had any effect on g_m or A . The $g_s - g_m$ relationship therefore may reflect a tight coordination between A and g_m , or a tendency for g_m to compensate for reductions in g_s . Both these patterns turn out to be predicted by optimisation, as discussed below.

For canopy-level modelling, the effects of environmental factors (light, $[CO_2]$, etc.) on g_m are important. Recent reviews have already highlighted the incidence of varying environmental conditions on g_m (Flexas et al. 2012; Warren 2008b), and so what we review here are short- and longer-term responses of g_m to light because these are key to canopy-scale modelling of the economics of N and water use in photosynthesis. Several studies have reported a positive relationship between g_m and short-term (minutes) changes in irradiance (Flexas et al. 2007; Hassiotou et al. 2009; Douthe et al. 2012; Douthe et al. 2011). There are presently too few data to comment on the exact shape of the g_m light-response, although, as a first approximation, it would appear that at least in *Eucalyptus*, the relationship is linear at low light intensities but, like the response of net photosynthesis, saturates at moderate light intensities (Douthe et al. 2011). Interestingly, the response of g_m to light in *Eucalyptus* led to a very stable c_c (and $c_i - c_c$ draw down) across irradiance levels, despite large variation in A .

In the longer term g_m acclimates to the light environment and thus varies among leaves in a canopy. Trends in

g_m , g_s and A between sun and shade leaves are rather inconsistent and vary among studies—even between studies on the same species. In some cases, differences in g_m between sun and shade leaves are in the same direction as those in photosynthesis and stomatal conductance (Warren et al. 2007; Yamori et al. 2006) and the approximate scaling of g_m with photosynthetic capacity means that the CO_2 drawdown, $c_i - c_c$, generally varies much less than A across light gradients (Piel et al. 2002; Warren et al. 2003; Warren et al. 2007). In other cases, however, g_m does not scale with g_s and A . For example, in *Fagus sylvatica* g_m was reported to be smaller in upper canopy leaves than mid canopy leaves and the ratio g_m/g_s decreased with height (Bögelein et al. 2012; Montpied et al. 2009). Similarly, in one study with *Pseudotsuga menziesii*, the degree of limitation of photosynthesis by g_m was somewhat larger in sun leaves (Niinemets et al. 2006), whereas in another study comparing sun and shade leaves of *Pseudotsuga menziesii* g_m scaled with g_s and A (Warren et al. 2003).

How does variation in g_m affect photosynthetic resource economics?

Several features of the variations and responses of g_m as outlined above may impact on nitrogen, water and carbon economy of leaves and canopies. One such feature is the possible nitrogen costs associated with g_m : i.e., enzymes or membrane proteins that may contribute to g_m . Another feature is the short-term responses of g_m to environment, notably irradiance, CO_2 , temperature, drought and vapour pressure deficit. A third is the observation that g_m often appears to face physiological limitations that prevent indefinite increases in g_m in upper canopy leaves. The discussion here will touch on the economic dimensions of each of these three features.

How should g_m vary in relation to g_s , photosynthetic capacity and environment?

It is useful to ask how g_m would vary if plants could manipulate it without constraints. Since g_m represents a constriction on supply of a limiting resource, CO_2 , to the photosynthetic apparatus, it seems obvious that g_m should simply be infinite unless it is constrained either by a physiological limitation or a resource tradeoff, or both. Yet, as discussed above, this is not the case: g_m varies widely among leaves and even over time in response to environmental conditions. Therefore, it is likely that g_m is limited at least in some instances either by a fundamental biophysical limit, perhaps involving anatomy (Nobel 1977), or by resource investment, perhaps N for building aquaporins, carbonic anhydrase or other proteins that contribute to g_m ,

and ongoing costs of maintaining such proteins (Cowan 1986).

In order to explore the economic implications of these two scenarios—physiological limits to g_m or nitrogen costs of g_m —it is first useful to recap the logic and mathematics of optimal water and photosynthetic N use in canopies. PNUE and WUE typically increase monotonically as photosynthetic N content or conductance, respectively, decrease, so maximising PNUE or WUE leads to a trivial solution of zero resource investment. Therefore, the question of optimal water and N use is better framed as a constrained optimisation problem: assuming the plant has finite supplies of water and N, how should they be distributed in space and over time (and, for N, among functional pools) to maximise carbon gain? The abstract solution to this problem is that the marginal carbon products of water and nitrogen use, $\partial A/\partial E$ and $\partial A_d/\partial N$, should be invariant—among leaves and over time for water, and among leaves and among functional pools within leaves for N (Buckley et al. 2002; Cowan 1977; Farquhar 1989; Field 1983). (Note that A_d is average or total assimilation rate over a day.) The target values of these marginal products are often denoted $1/\lambda$ for water and $1/\nu$ for nitrogen. What do these abstract solutions imply for actual distributions of N and water use? In general, photosynthetic capacity and stomatal conductance should increase with irradiance (Cowan 1977; Sands 1995; Farquhar 1989). Simple models predict a linear and homogeneous relationship between photosynthetic N (N invested in the photosynthetic apparatus, including Rubisco and electron transport) and irradiance, but observations suggest the relationship is rather scattered and has negative curvature (i.e. photosynthetic capacity per unit irradiance declines at high light) (Niinemets 2012). A recent modelling study (Buckley et al. 2013) suggests the scatter results at least in part from averaging over a complex, dynamic and multimodal light environment (i.e. sunflecks). The same study also found that negative curvature in the optimal capacity vs irradiance relationship should result from anything that systematically reduces CO₂ supply to the mesophyll at higher irradiance, such as height-related declines in stomatal conductance or physiological limits to the ability of g_m to track photosynthetic capacity at high light (Niinemets et al. 2006). We explore later why this should be the case, and we ask further how g_m should vary with photosynthetic capacity if g_m is in fact limited by N investment.

Economic impact of a nitrogen cost for g_m

We begin by exploring the implications for scaling of g_m to photosynthetic capacity if g_m has a nitrogen cost, such that g_m increases monotonically with some functional N pool, say N_m . How then should N investment in g_m scale remain

in relation to investment in Rubisco? When photosynthesis is Rubisco limited, the marginal C product of N_m can be shown to be (see the Appendix)

$$\frac{\partial A}{\partial N_m} = \frac{kA}{g_m^2} \left(\frac{g/k}{g/k + 1} \right) \frac{\partial g_m}{\partial N_m} \tag{1}$$

where k is the slope of the A versus c_c demand curve at the operating point (the intersection of the supply and demand curves), and g is total conductance to CO₂. (Note that we use instantaneous A in this discussion to allow derivation of analytical expressions, whereas the marginal product for N should use the daily average or integral of A .) In the optimum, $\partial A/\partial N$ must be equal for all N pools, including Rubisco N. Therefore, we will set Eq. 1 equal to the marginal C product of Rubisco N (N_v), which under Rubisco-limited conditions is given by (see the Appendix)

$$\frac{\partial A}{\partial N} = \frac{\partial A}{\partial N_v} = \frac{A}{N_v} \left(\frac{g/k}{g/k + 1} \right) \tag{2}$$

If we set Eqs. 1 and 2 equal, apply an expression for k under Rubisco limited conditions, and finally solve for the ratio of g_m to V_m , then we obtain the following expression for the optimal coordination of N investments in mesophyll conductance and carboxylation capacity:

$$\frac{N_m}{N_v} = \sqrt{\frac{\chi_v (\Gamma_* + K')}{\chi_m (c_c + K')}} \tag{3}$$

where K' is the effective Michaelis constant for RuBP carboxylation, Γ_* is the photorespiratory CO₂ compensation point, and χ_m is the proportionality between N_m and that part of g_m that depends on N_m . The result in Eq. 3 applies whether g_m is simply proportional to N_m (so that $g_m = \chi_m N_m$ with χ_m a constant) or if, instead, g_m represents two diffusion pathways in series, one of which depends on N_m and the other on Rubisco N (so that $g_m = (\chi_m N_m)(\chi_{mv} N_v)/(\chi_m N_m + \chi_{mv} N_v)$, with χ_m and χ_{mv} both being constants). (The latter case could arise if, for example, investment in Rubisco leads to an increase in chloroplast surface area, which could increase g_m .) Thus, in these scenarios, the optimal scaling between mesophyll conductance N and Rubisco N is determined primarily by c_c . Anything that reduces c_c systematically up through the canopy—for instance, height-related limitations on stomatal conductance arising from the need to limit transpiration, and to prevent runaway xylem embolism—should favour a shift of N from Rubisco to mesophyll conductance. This is consistent with the results of Pons and Welschen (2003) discussed above, in which g_m increased when g_s declined due to midday depression. We note that Eq. 3 does not apply if g_m responds nonlinearly to the increase in N_m or Rubisco N; this case, and others in which the effect of N on g_m is more complicated than assumed

above, could be assessed within this framework but would likely require extension of the model and numerical solution.

Economic impact of physiological constraints on g_m

We now ask how optimal water and photosynthetic N use should be affected by physiological constraints on g_m . Suppose g_m ‘tracked’ g_s perfectly, such that $g_m = \gamma g_s$ with γ an invariant parameter among leaves and over time. Then, g_m would have no *systematic* impact on economics of N and water use, because it would be mathematically indistinguishable from a uniform proportional decrease in stomatal conductance everywhere: namely, total conductance g would decline from $g = g_s$ to $g = g_s g_m / (g_s + g_m) = g_s / (1 + \gamma)$, which is equivalent to g_s declining by a factor $\gamma / (1 + \gamma)$ (this ignores boundary layer resistance for simplicity). Therefore, if short-term responses of g_m to irradiance, CO₂ and drought simply paralleled those of g_s , then g_m would have no systematic effect on photosynthetic resource economics.

However, as discussed earlier, these responses often do not closely parallel one another. The economic impact of physiological constraints on g_m , or more generally of divergence in the responses of g_m and g_s to environmental parameters, should thus be compared against a default scenario in which γ is invariant among leaves. We ask how the optimal values of g_s and photosynthetic capacity in any particular leaf are affected by a decrease in g_m relative to g_s (i.e. by a decrease in γ for a given g_s). To answer this question, we will first derive implicit relationships among N , g_s and γ in the optimum, and then deduce from these relationships how N and/or g_s must adjust in response to a small perturbation in γ to re-establish the ‘target’ values of $\partial A / \partial N$ and $\partial A / \partial E$.

First, we identify the direction in which N must change. Equation 2 shows that the marginal return on N investment in Rubisco increases with the ratio of CO₂ supply to demand— g/k (more precisely, the ratio of the slopes of the CO₂ supply and demand curves). Suppose now that a particular leaf, perhaps an upper-canopy leaf, is constrained to a smaller g_m (relative to g_s) than other leaves in the same canopy. In other words, γ is lower for this leaf. This decrease in γ has three effects on $\partial A / \partial N$. First, it directly decreases g . This decreases c_c , which in turn causes the second and third effects: a decrease in A and, because the operating point moves back down the demand curve to a steeper region, an increase in k . Each of these three effects reduces the supply/demand ratio (g/k) and thus $\partial A / \partial N$. To re-establish the target value of $\partial A / \partial N$ and thereby maintain optimal N distribution among leaves in this canopy, photosynthetic N must decline in this low- γ leaf. To verify this logic and provide a visual

demonstration, we also performed calculations using a gas exchange model (described in the Appendix). Figure 1 shows how c_c , k , A and $\partial A / \partial N$ vary with γ if g_s is held constant in that model. These calculations indicate that $\partial A / \partial N$ declines if γ is reduced, confirming the analysis above.

We now identify the direction in which g_s must change. We show in the Appendix that

$$\frac{\partial A}{\partial E} = \frac{A}{E} \left(\frac{k/g}{k/g+1} \right) \left(\frac{\gamma}{\gamma+1} \right) \quad (4)$$

In this case, the marginal product responds to the ratio of demand to supply, k/g , and also to γ itself. How does a decline in γ affect this relationship? Both A and γ itself decline, whereas k/g increases and E is not affected, and so

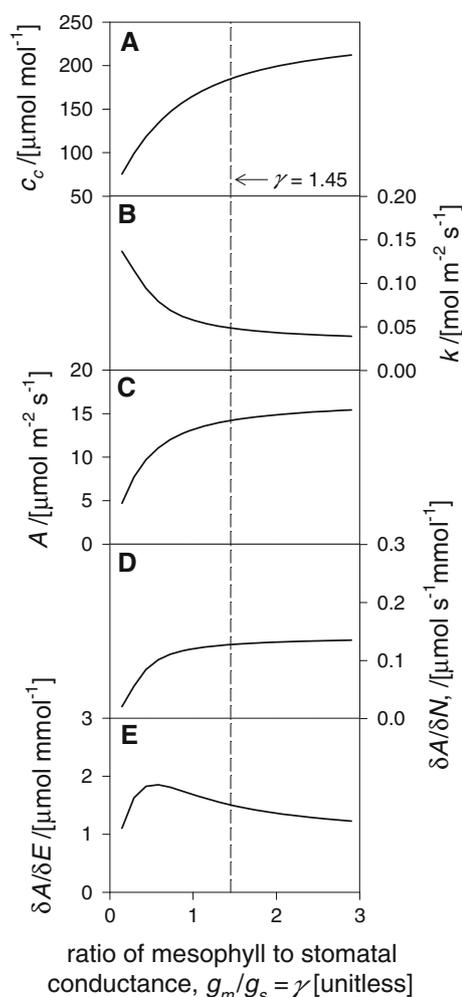


Fig. 1 Effect of variation in mesophyll conductance, g_m , expressed in proportion to stomatal conductance, g_s , in the ratio γ , on variables related to analysis of N and water economy. **a** chloroplastic CO₂ mol fraction, c_c . **b** slope of the demand curve, k . **c** net CO₂ assimilation rate, A . **d** the marginal carbon product of N, $\partial A / \partial N$. **e** the marginal carbon product of water, $\partial A / \partial E$. The dashed line is the value of γ (1.45) used as the initial condition in Fig. 2. See the Appendix for further details

it is not obvious from inspection whether $\partial A/\partial E$ increases or decreases. However, our numerical calculations suggest that $\partial A/\partial E$ generally increases if γ declines (Fig. 1). Therefore, in order to counteract these changes and re-establish the target value of the marginal product, stomatal conductance must adjust so as to reduce $\partial A/\partial E$. This requires an increase in g_s .

Thus, an inspection of Eqs. 2 and 4 suggests that a reduction in g_m relative to g_s requires an increase in g_s and a decrease in N to re-establish the optimal state. However, these changes do not occur in isolation from one another: g_s affects $\partial A/\partial N$ and N affects $\partial A/\partial E$. These second-order effects are difficult to deduce by inspection, and so we assessed them numerically. Specifically, we computed $\partial A/\partial E$ and $\partial A/\partial N$ for an initial state, and then we decreased γ by half and adjusted g_s and N iteratively to re-establish the values of $\partial A/\partial E$ and $\partial A/\partial N$ that had been calculated in the initial state. (Note that we held γ , rather than g_m itself, constant during these adjustments of g_s , so that g_m was also implicitly varying.) We found that it was necessary to increase g_s by 14 % and decrease photosynthetic N by 3 % to re-establish the initial values of the marginal products (Fig. 2). We also found that it was impossible to re-establish these initial values by changing either g_s or N alone.

Implications of these predictions from optimisation

Several points arise in relation to these predictions. First, they apply not only to longer-term acclimation of leaves to, for example, irradiance regimes (Piel et al. 2002; Warren et al. 2003; Warren et al. 2007; Bögelein et al. 2012; Montpied et al. 2009) or tree height (Whitehead et al. 2011; Han 2011; Woodruff et al. 2009), but also to short-term differences in the relative responses of g_s and g_m to environmental conditions (e.g. in response to water stress: Galmes et al. 2011; Duan et al. 2009). For example, suppose g_m cannot respond as sensitively to irradiance as g_s can. This will cause γ to decline with increasing irradiance

in a given leaf, such that optimal g_s will be greater at high light than it would be if it were physiologically possible for g_m to ‘track’ g_s perfectly.

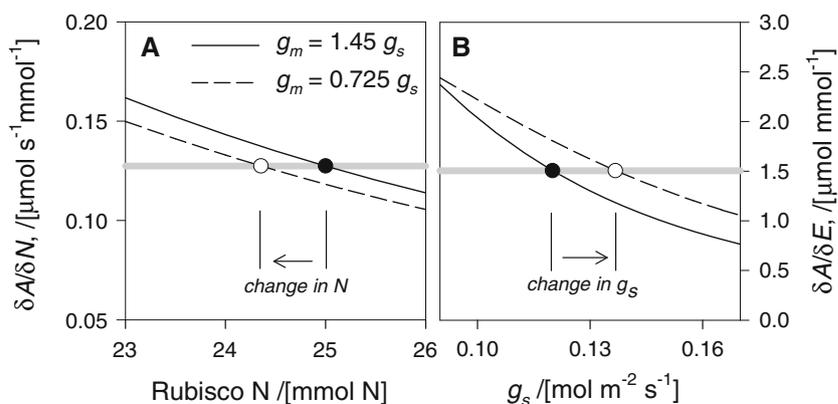
Second, because constraints on g_m require a compensatory increase in g_s , the result is that c_i goes up while c_c goes down. This means that the drawdown ($c_i - c_c$) increases. This prediction also arose in much more detailed simulations by Buckley et al. (2013) (Fig. 3), which account for integration over a day and for many other considerations omitted in the simple analysis here. We are unaware of any data showing c_i increasing up through the canopy, and so this prediction suggests that, if indeed g_m is constrained and g_s increases to compensate, this effect is more than offset by some other factor that reduces g_s , for example, reduced water potential in upper canopy leaves.

Third, empirical measurements of g_m that are currently available provide qualified support, but no firm conclusions about whether the predictions outlined above hold up empirically. In canopies of *Fagus sylvatica*, g_m is lower in upper canopy leaves than in mid-canopy leaves, but there is little difference in total conductance to CO₂ transfer due to ‘compensatory’ increases in g_s (Bögelein et al. 2012; Montpied et al. 2009). However, the more general trend is for g_m and g_s to vary in parallel. For example, g_m and g_s are commonly (but not always) higher in upper canopy leaves than in lower canopy leaves (Piel et al. 2002; Warren et al. 2003; Warren et al. 2007). Similarly, in comparisons of trees of differing height, g_m and g_s are commonly smaller in tall trees than in short trees (Han 2011; Woodruff et al. 2009; Whitehead et al. 2011).

Summary: directions for research on the economics of mesophyll conductance

It is reasonable to surmise that g_m is actively controlled by plants given evidence showing g_m is plastic at a range of time scales and varies non-randomly within tree canopies according to, *inter alia*, gradients in light. The obvious

Fig. 2 Demonstration of simultaneous changes in Rubisco N (N_v) and stomatal conductance (g_s) required to re-establish initial values (thick grey lines) of the marginal carbon products of N ($\partial A/\partial N$, a) and water ($\partial A/\partial E$, b), if mesophyll conductance (g_m) were reduced by half, from $1.45 \cdot g_s$ (solid lines) to $0.725 \cdot g_s$ (dashed lines). Calculations are described in the Appendix



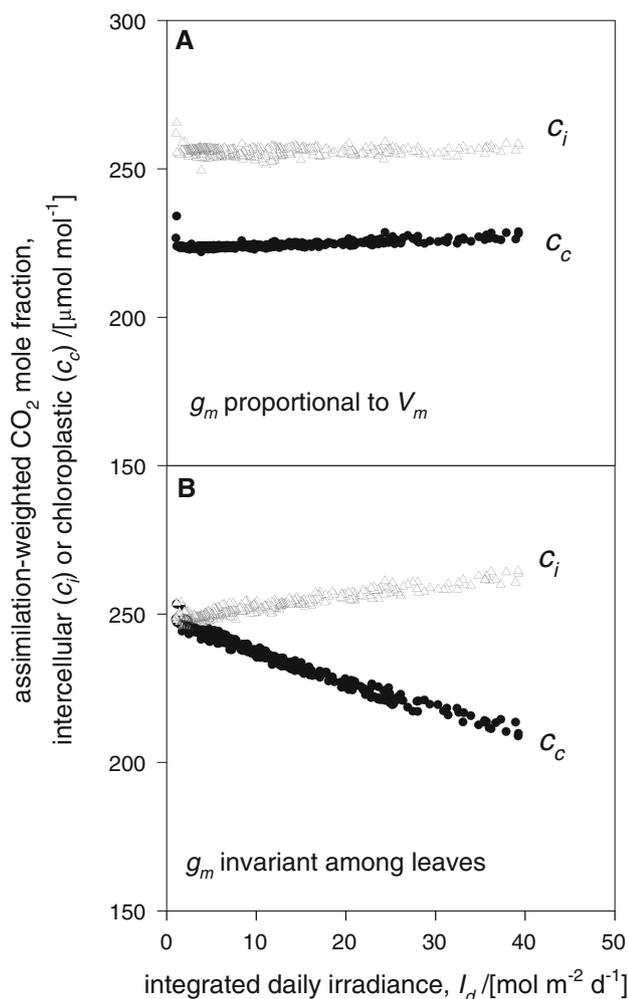


Fig. 3 Variation in intercellular and chloroplastic CO₂ mol fractions (c_i and c_c , respectively) among leaves in relation to integrated daily incident irradiance, when photosynthetic nitrogen and stomatal conductance are optimised in a detailed crown model, assuming **a** mesophyll conductance, g_m , is proportional to carboxylation capacity, V_m ; or **b** g_m is invariant among leaves. Redrawn from Buckley et al. (2013)

questions are why and how plants modulate g_m . Optimisation of N and water in terms of photosynthesis has proved enormously powerful in terms of explaining trends in A and g_s through canopies (Farquhar 1989; Field 1983; Niinemets 2012; Peltoniemi et al. 2012; Sands 1995). It stands to reason that the same logic might be useful for exploring why g_m does (or does not) vary. The brief theoretical analysis in this article suggests that the major regulatable controls on CO₂ supply and demand— g_m , g_s and photosynthetic capacity—should mutually adjust so as to compensate for local biophysical constraints on one or more of these controls, thereby re-establishing the balance between supply and demand that optimisation requires to remain uniform throughout the canopy. For example, if g_m

is limited in the upper canopy, then photosynthetic N should decline and stomatal conductance should increase in the upper canopy, decreasing CO₂ demand relative to supply. Similarly, if g_s is limited in the upper canopy, and if g_m is in fact limited by N investment in some functional pool, then N should shift to that pool and away from photosynthetic capacity.

Various studies have investigated how g_m varies through canopies, but have not determined marginal carbon products of N and water use; thus, we remain generally incapable of rigorously testing predictions of optimisation theory. We suggest two lines of approach to redress this limitation and move forward. First, fundamental experimental research is still needed to clarify the physiological origin of g_m , and particularly its possible nitrogen costs, so that g_m can be placed on equal economic footing as g_s and photosynthetic capacity. Second, careful definitive tests of the predictions of optimisation theory for patterns in g_m are needed. Such tests must accurately measure the variables that determine marginal carbon products of N and water use— g_m , g_s , photosynthetic capacity, and key environmental variables including irradiance, evaporative demand and leaf temperature—and apply them to a model to calculate those marginal products, at the same time that supply and demand for CO₂ are independently manipulated in different canopy layers—e.g. by reducing or increasing g_s , irradiance and [CO₂]. This would disentangle effects of CO₂ supply and demand on g_m while also controlling for factors that confound observational studies (i.e. the many other factors that vary with height in the canopy) and placing variations in g_m into the spatiotemporal context where the question of resource allocation is relevant and can be addressed. Previous studies have shown that independent and reversible manipulations of supply and demand are feasible with small seedlings and can provide novel insights into controls of photosynthesis and transpiration (Pepin et al. 2002). The challenge will be to do conceptually similar experiments while quantifying g_m .

Appendix

Analytical expressions for marginal carbon products of N and water use

The marginal carbon product of photosynthetic N, $\partial A/\partial N$, was given by Buckley et al. (2002) as

$$\frac{\partial A}{\partial N} = \left(\frac{\partial A}{\partial N} \right)_{c_c} \left(\frac{g}{g+k} \right) \quad (\text{A1})$$

where the partial derivative at right, $\partial A/\partial N$ at constant c_c , is

$$\left(\frac{\partial A}{\partial N}\right)_{c_c} = (A + R_d) \frac{\partial \ln W}{\partial N} - \frac{R_d}{N} \tag{A2}$$

In A2, W represents the maximum RuBP carboxylation velocity, V_m , when photosynthesis is carboxylation limited, or the potential electron transport rate, J , when photosynthesis is RuBP regeneration limited; and R_d is non-photorespiratory CO_2 release concurrent with photosynthesis. These derivatives apply to components of N as well, including Rubisco N (N_v), so N can be replaced with N_v in Eq. A2. Then, if $V_m = \chi_v N_v$, $\partial \ln V_m / \partial N_v = 1/N_v$ and

$$\frac{\partial A}{\partial N_v} = \frac{A}{N_v} \left(\frac{g}{g+k}\right) \tag{A3}$$

Equation 2 in the main text is obtained by dividing through by k in the term in parentheses in Eq. A3. The marginal C product of water use, $\partial A / \partial E$, under well-coupled conditions (negligible boundary layer resistance and constant temperature) is found by applying Eqs. A6, A18 and A23 to Eq. A40 in Buckley et al. (2002) to give

$$\frac{\partial A}{\partial E} = \frac{A}{E} \left(\frac{k}{k+g}\right) \frac{1.6g}{g_w} \tag{A4}$$

where g_w is total conductance to H_2O . Under well-coupled conditions $g = g_s \cdot g_m / (g_s + g_m) = g_s \cdot \gamma / (\gamma + 1)$ (where $\gamma = g_m / g_s$) and $g_w = 1.6 g_s$. Then, $1.6g / g_w = \gamma / (\gamma + 1)$. Equation 4 in the main text arises by applying this result to A4 and dividing through by g in the term in parentheses.

Analytical expression for marginal C product of mesophyll conductance N

If we hypothesise that mesophyll conductance increases monotonically in relation to the size of some N pool, N_m , then the marginal C product of N_m , $\partial A / \partial N_m$, is

$$\frac{\partial A}{\partial N_m} = \frac{\partial A}{\partial c_c} \frac{\partial c_c}{\partial r_m} \frac{\partial r_m}{\partial g_m} \frac{\partial g_m}{\partial N_m} \tag{A5}$$

where $r_m = 1/g_m$. The first partial on the right-hand side, $\partial A / \partial c_c$, is the slope of the demand curve, k , at the operating point, and the third is $-1/g_m^2$. The second, $\partial c_c / \partial r_m$, is found by differentiating the equation of CO_2 diffusion with respect to c_c . Ignoring boundary layer resistance as above and writing $r_s = 1/g_s$, the diffusion equation is $A = (c_a - c_c) / (r_s + r_m)$, which rearranges to $c_c = c_a - r_m A - r_s A$. Then,

$$\frac{\partial c_c}{\partial r_m} = -A - (r_m + r_s) \frac{\partial A}{\partial r_m} = -A - \frac{1}{g} \frac{\partial A}{\partial r_m} \tag{A6}$$

Noting that $\partial A / \partial r_m = (\partial A / \partial N_m) / [(\partial r_m / \partial g_m)(\partial g_m / \partial N_m)]$ and applying this and Eq. A6 to A5, we have

$$\frac{\partial A}{\partial N_m} = k \left(-A \frac{\partial r_m}{\partial g_m} \frac{\partial g_m}{\partial N_m} - \frac{1}{g} \frac{\partial A}{\partial N_m} \right) = \frac{\partial g_m}{\partial N_m} \frac{k}{g_m^2} A - \frac{k}{g} \frac{\partial A}{\partial N_m} \tag{A7}$$

This is readily solved for $\partial A / \partial N_m$ to give

$$\frac{\partial A}{\partial N_m} = \frac{\partial g_m}{\partial N_m} \frac{k}{g_m^2} A \left(\frac{g}{g+k}\right) \tag{A8}$$

From Eq. A25 in Buckley et al. (2002), k under Rubisco-limited conditions is

$$k = V_m \frac{(\Gamma_* + K')}{(c_c + K')^2} \tag{A9}$$

Expanding V_m as $\chi_v N_v$, applying A9 to A8 and rearranging gives the ratio of g_m to V_m in the optimum as

$$\frac{g_m}{V_m} = \sqrt{\frac{1}{\chi_v} \frac{\partial g_m}{\partial N_m} \frac{(\Gamma_* + K')}{(c_c + K')^2}} \tag{A10}$$

If we assume g_m is directly proportional to N_m , say $g_m = \chi_m N_m$, then $\partial g_m / \partial N_m = \chi_m$, so

$$\frac{g_m}{V_m} = \sqrt{\frac{\chi_m (\Gamma_* + K')}{\chi_v (c_c + K')^2}} \tag{A11}$$

or equivalently, in terms of the ratio of N pools,

$$\frac{N_m}{N_v} = \sqrt{\frac{\chi_v (\Gamma_* + K')}{\chi_m (c_c + K')^2}} \tag{A12}$$

which is Eq. 3 in the main text. If instead g_m represents two diffusion pathways in series, one of which scales with N_m and the other of which scales with N_v (with proportionality constant χ_{mv}), then

$$g_m = \frac{(\chi_m N_m)(\chi_{nv} N_v)}{\chi_m N_m + \chi_{nv} N_v} \tag{A13}$$

and $\partial g_m / \partial N_m$ is $g_m^2 / (\chi_m N_m^2)$. Applying this to A8 and rearranging leads to the same expression as given in A12. Therefore, A12 applies whether g_m is given by $\chi_m N_m$ or by Eq. A13.

Numerical calculations for Figs. 1 and 2

We calculated $\partial A / \partial N$ and $\partial A / \partial E$ from the photosynthesis model of Farquhar et al. (1980) as described by Buckley et al. (2002), assuming zero boundary layer resistance. Net CO_2 assimilation rate was calculated from two rates, one applying in RuBP carboxylation limited conditions (A_v), and the other in RuBP regeneration limited conditions (A_i):

$$A_v = \frac{V_m (c_c - \Gamma_*)}{c_c + K'} - R_d \tag{A14}$$

$$A_j = \frac{1}{4} \frac{J(c_c - \Gamma_*)}{c_c + 2\Gamma_*} - R_d \quad (\text{A15})$$

and A was taken as the smaller root of $\theta_A A^2 - A(A_v + A_j) + A_v A_j = 0$ where θ_A is a dimensionless curvature parameter (0.99). The intersection of this solution with an expression for CO_2 diffusion to the sites of carboxylation, $A = g(c_a - c_c)$, leads to a quartic expression for c_c , which is then substituted into the diffusion equation to calculate A . The potential electron transport rate J was taken as the smaller root of $\theta_A J^2 - J(J_m + \phi I) + J_m \phi I = 0$, where J_m is maximum potential electron transport rate (taken as $2.1V_m$, Wullschlegel 1993); I is incident irradiance ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$); ϕ is effective maximum quantum yield of electrons from incident irradiance ($0.25 e^-/\text{hv}$); and θ_j is a dimensionless curvature parameter (0.86). Other parameters were as follows: effective Michaelis constant for RuBP carboxylation, K' , $617 \mu\text{mol mol}^{-1}$; photorespiratory CO_2 compensation point, Γ_* , $37 \mu\text{mol mol}^{-1}$; ambient CO_2 mol fraction, c_a , $385 \mu\text{mol mol}^{-1}$; and respiration rate in the light, R_d , $0.01V_m$. These values for K' and Γ_* are approximately equivalent to a temperature of 25°C and normal atmospheric pO_2 (Sharkey et al. 2007).

V_m was taken as $4.5 \cdot N_v$; this proportionality arises from $6,290 \text{ mol N per mol of Rubisco}$ (Hikosaka and Terashima 1995), a turnover time of 3.53 s^{-1} (von Caemmerer and Evans 1991), and eight active sites per Rubisco molecule. Default values for N_v , g_s and γ (g_m/g_s) were 25 mmol m^{-2} , $0.12 \text{ mol m}^{-2} \text{ s}^{-1}$ and 1.45 , respectively. The value for γ is the grand mean of $(c_a - c_i)/(c_i - c_c)$, the ratio of the CO_2 drawdowns from the ambient air to the intercellular air spaces and from the intercellular spaces to the sites of carboxylation, given in Table 2. The default values for N_v and g_s were chosen arbitrarily to give realistic values of c_i and c_c . Calculation of $\partial A/\partial E$ also requires a value for leaf to air water vapour mole fraction gradient, which we took as 20 mmol mol^{-1} . The response curves in Figs 1 and 2 were generated by varying these quantities about these default values (the latter are represented by the dashed line in Fig. 1 and by solid symbols in Fig. 2).

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