

How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress



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ABSTRACT

Models disagree on how to represent effects of drought stress on plant gas exchange. Some models assume drought stress affects the marginal water use efficiency of plants (marginal WUE = $\partial A / \partial E$; i.e. the change in photosynthesis per unit of change in transpiration) whereas others assume drought stress acts directly on photosynthetic capacity. We investigated drought stress in an analysis of results from 22 experimental data sets where photosynthesis, stomatal conductance and predawn leaf water potential were measured at increasing levels of water stress.

Our analysis was framed by a recently developed stomatal model that reconciles the empirical and optimal approaches to predicting stomatal conductance. The model has single parameter g_1 , a decreasing function of marginal WUE. Species differed greatly in their estimated g_1 values under moist conditions, and in the rate at which g_1 declined with water stress. In some species, particularly the sclerophyll trees, g_1 remained nearly constant or even increased.

Photosynthesis was found almost universally to decrease more than could be explained by the reduction in g_1 , implying a decline in apparent carboxylation capacity (V_{cmax}). Species differed in the predawn water potential at which apparent V_{cmax} declined most steeply, and in the steepness of this decline. Principal components analysis revealed a gradient in water relation strategies from trees to herbs. Herbs had higher apparent V_{cmax} under moist conditions but trees tended to maintain more open stomata and higher apparent V_{cmax} under dry conditions. There was also a gradient from malacophylls to sclerophylls, with sclerophylls having lower g_1 values under well-watered conditions and a lower sensitivity of apparent V_{cmax} to drought.

Despite the limited amount of data available for this analysis, it is possible to draw some firm conclusions for modeling: (1) stomatal and non-stomatal limitations to photosynthesis must both be considered for the short-term response to drought and (2) plants adapted to arid climate respond very differently from others.

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1. Introduction

Soil water deficit or “ecological drought” is considered to be the main environmental factor limiting global plant photosynthesis (Nemani et al., 2003). Modeling the effect of drought on photosynthesis (A) and stomatal conductance (g_s) is crucial to understand and project the consequences of global environmental change for plants and ecosystems. However, there is disagreement among models in how to represent drought effects. Many models simply reduce the slope of the g_s/A relationship (e.g. Battaglia et al.,

2004; Kirschbaum, 1999; Friend and Kiang, 2005; Medlyn, 2004; Sala and Tenhunen, 1996; Wang and Leuning, 1998), whereas others assume drought affects A directly by reducing V_{cmax} (maximum rate of carboxylation) and/or J_{max} (maximum rate of electron transport) in the Farquhar et al. (1980) C_3 photosynthesis model (e.g. Calvet et al., 2004; Keenan et al., 2009; Krinner et al., 2005; Moorcroft et al., 2001; Sellers et al., 1996). Only a few models include both effects (e.g. the Sheffield Dynamic Global Vegetation Model, SDGVM) (Woodward and Lomas, 2004). Recent studies have suggested that both effects ought to be included (Egea et al., 2011), but it is not known which approach best captures the drought response, nor is it known how drought responses vary among species and plant functional types (PFTs). The goal of this paper is to investigate drought responses in a range of species. Datasets of photosynthesis, stomatal conductance and pre-dawn leaf water

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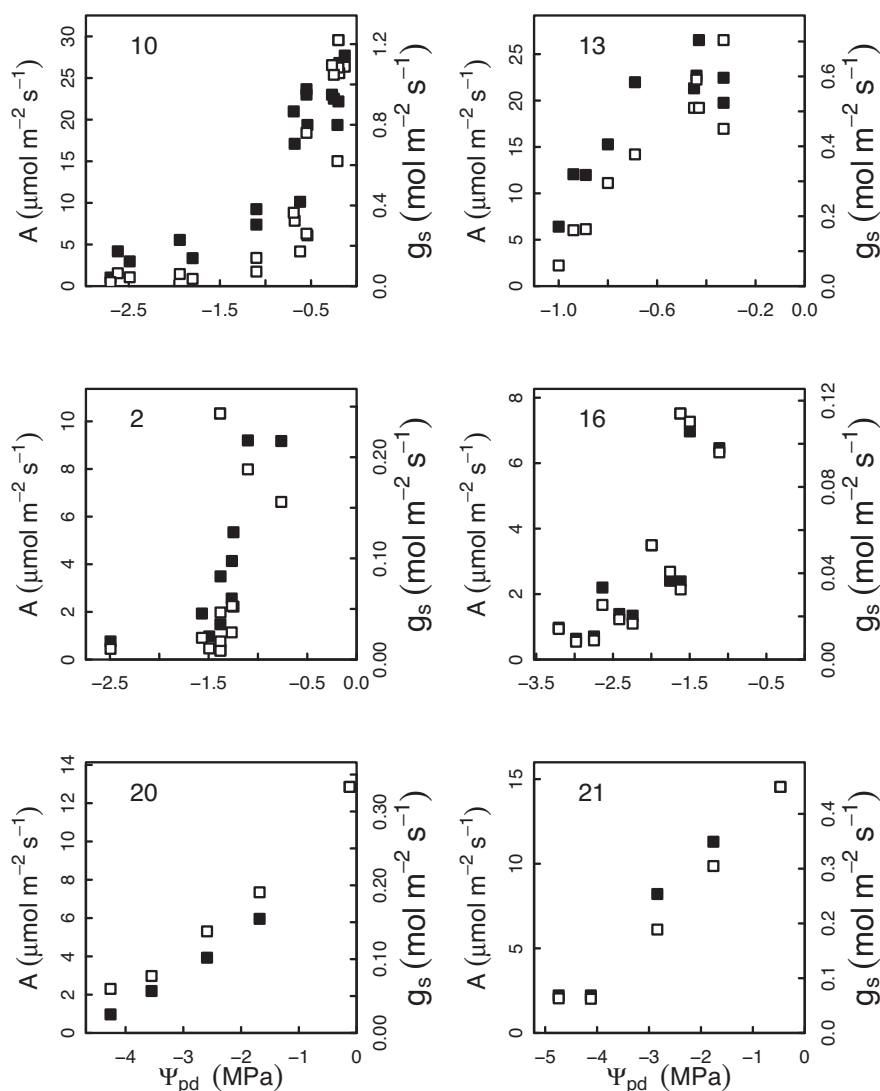


Fig. 1. A (filled squares) and g_s (open squares) responses to Ψ_{pd} , from two data sets representative for each of three PFTs. Herbs: (10) *Helianthus annuus* and (13) *Mediterranean Herbs*; Malacophyll angiosperm tree: (2) *Broussonetia papyrifera* and (16) *Platycarya longipes*; Sclerophyll angiosperm tree: (20) *Quercus ilex* and (21) *Quercus suber*.

potential during drying cycles were obtained from the literature (Fig. 1.) and were analyzed in the framework of a model of optimal stomatal conductance.

The theory of optimal stomatal behavior has been influential in explaining how carbon gain and water loss are balanced. Optimality theory hypothesizes that plants regulate stomatal opening and closing in such a way as to maximize $(A - \lambda E)$ where A is photosynthesis, E is transpiration, and λ is the marginal carbon cost of water to the plant (Cowan, 1977; Cowan and Farquhar, 1977). When water availability decreases, it is hypothesized that λ increases, due to the risk of damage from hydraulic failure if plants maintain high transpiration rate, and/or the increased cost of building structures that are more hydraulically efficient (Berninger and Hari, 1993). Theoretical analysis by Mäkelä et al. (1996) indicated that $1/\lambda$ should be expected to decline exponentially with decreasing soil moisture availability, and the rate of decline with soil moisture should increase with the probability of rain.

We use the term stomatal limitation to refer to this idea that the optimal stomatal conductance declines in response to drought causing a decline in photosynthesis. There can also be non-stomatal limitation of photosynthesis, which involves a reduction in apparent V_{cmax} . If A declines with drought more steeply than can be explained by the observed stomatal limitation, this indicates the

presence of non-stomatal limitation. Thus we interpret stomatal limitation as involving a change in the leaf-internal concentration of CO_2 (C_i) and non-stomatal limitation as a change in the $A-C_i$ curve (Fig. 2). Note that our approach differs from one traditional way of analysing the drought effect on photosynthesis in terms of stomatal and non-stomatal limitations, using the equations from Jones (1985) (e.g. Grassi and Magnani, 2005; Keenan et al., 2009; Kubiske and Abrams, 1993; Ni and Pallardy, 1992; Wilson et al., 2000). Our method differs from that of Jones (1985) because the evidence for stomatal limitation is considered to be reduced C_i and not just reduced g_s , which could also arise a response to biochemical limitation. The difference is important because, in the optimal stomatal model and similar empirical models (e.g. Ball et al., 1987; Leuning, 1995), any reduction in apparent V_{cmax} will drive a reduction in stomatal conductance. Our approach partitions drought effects on stomatal conductance into changes that are an optimal response to a reduction in V_{cmax} , and reductions that are driven by an increase in the marginal cost of water. This way of thinking about stomatal conductance has the advantage of being able to be translated directly into modeling terms.

There has been controversy – perhaps fueled by this ambiguity over definitions – over the extent to which photosynthesis

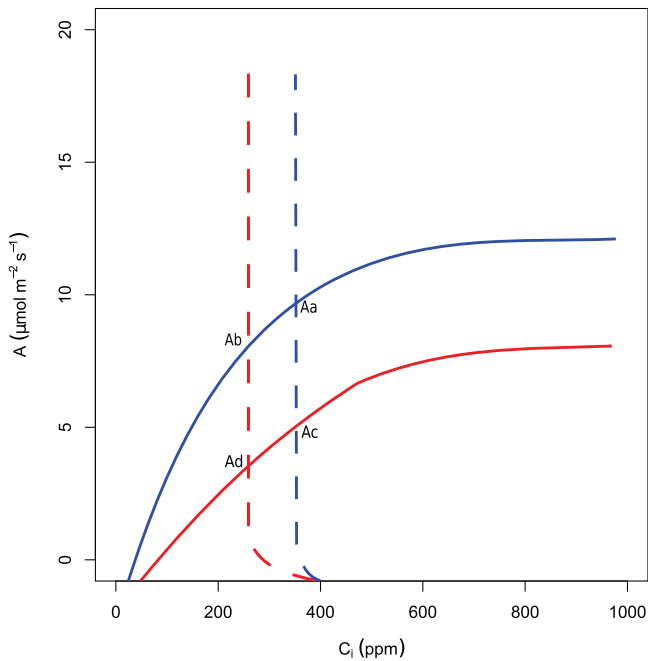


Fig. 2. Framework for the analysis of stomatal and non-stomatal limitations to photosynthesis. The non-stomatal limitation is represented by the solid lines, which shows the response of A to C_a . This curve depends on the apparent V_{cmax} . The stomatal limitation is represented by the dashed lines, which depend on g_1 . Blue lines represent well-watered conditions and red lines represent drought conditions. Under drought, a reduction in g_1 (stomatal limitation only) leads to a trajectory of A from Aa to Ab ; a reduction in apparent V_{cmax} (non-stomatal limitation only) leads to a trajectory from Aa to Ac ; and reduction in both parameters leads to a trajectory from Aa to Ad . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is more restricted by stomatal or non-stomatal limitations under drought conditions (e.g. Grassi and Magnani, 2005; Lawlor and Tezara, 2009). One strand of the literature has suggested that with increasing drought severity, non-stomatal limitations (due to either mesophyll conductance or leaf biochemistry or both) on photosynthesis come to predominate over stomatal limitation (e.g. Grassi and Magnani, 2005). Proposed non-stomatal mechanisms include reduced Rubisco activity (e.g. Medrano et al., 1997; Parry et al., 2002), reduced electron transport capacity (e.g. Cornic et al., 1989; Epron and Dreyer, 1992), and reduced mesophyll conductance (e.g. Egea et al., 2011); see Flexas et al. (2012) for a review. We refer to the concept of ‘apparent’ carboxylation capacity (V_{cmax}) recognizing that changes in V_{cmax} , when measured in the standard way, can arise either from changes in the actual capacity for carboxylation within the chloroplast, or from changes in g_m .

The objective of this work was to analyze available experimental data in a model-oriented framework in order to answer the question of how best to model the drought responses of gas exchange. In particular: (1) Which processes are affected? (2) What shape does the response function take? (3) How do the responses vary across species and hydro-climates? We have consistently used pre-dawn leaf water potential as the indicator of plant water stress (except one data set with only data on soil water potential). We investigated how the g_s/A relationship changes with experimental drought, and analyzed the potential role of changes in apparent V_{cmax} . The starting point for our study was the analysis by Manzoni et al. (2011), which describes marginal water use efficiency ($\partial A/\partial E$) as increasing monotonically with more negative leaf water potential (when finite cuticular conductance is taken into account). Our analysis was conducted in the framework of a recently derived and simple representation of the optimal stomatal model (Medlyn et al., 2011).

The model's single parameter g_1 is directly related to the marginal carbon cost of water (λ). To quantify the role of stomatal limitation, we examine the response of fitted values of g_1 to soil water deficit. To quantify the role of non-stomatal limitation, we examine the effect of water stress on apparent V_{cmax} , calculated from observed values of g_s and A .

2. Materials and methods

2.1. Sources of data

We analyzed 22 experimental data sets (two unpublished) that include concurrent measurement of net assimilation (A), stomatal conductance (g_s), and pre-dawn leaf water potential (Ψ_{pd}) (except two data sets on *Glycine max*: Huang et al., 1975 with soil water potential data and Liu et al., 2005 with root water potential data, which allow us to estimate Ψ_{pd}) spanning well-watered to water-stressed conditions. We confined our attention to studies that reported either Ψ_{pd} (except two allowing us to estimate Ψ_{pd}), because daytime leaf water potentials depend strongly on transpiration as well as soil water status. Ψ_{pd} is the best measure of water availability to the plant, since it integrates soil water potential over the root zone (Schulze and Hall, 1982) and is not influenced by daytime transpiration. It is also independent of soil texture, unlike volumetric soil moisture content, enabling us to compare species from experiments using different soil types.

Fourteen data sets were derived from Manzoni et al. (2011) via tables and digitized figures in the original sources. Six data sets were derived from two studies on species from karst substrates in southwest China. Two unpublished data sets were added, derived from drought experiments on two *Eucalyptus* species conducted in glasshouses at Macquarie University in 2011 (Kelly, unpublished data).

Most studies were under controlled conditions with plants growing in pots. Water stress was manipulated by withholding irrigation, and leaf gas exchange was measured on the same plant during a single drying cycle. Data on atmospheric CO_2 concentration (C_a) and vapor pressure deficit (D) were also derived from the reports. For a few studies where D was not reported, we assumed $D = 2$ kPa (Peguero-Pina et al., 2009), or $D = 2.1$ kPa (Liu et al., 2010). These D values are consistent with the sites' climate during the measurement period. We assumed dark respiration (R_d) to be negligible for our purpose. As a test of this assumption we estimated R_d as 7% of maximum photosynthesis rate recorded in each data set (Givnish, 1988), and found that this had a negligible effect on the results. The database spans tropical to boreal species, and herbaceous annuals to woody gymnosperms and angiosperms. Thus, the species are broadly representative of the PFTs commonly employed in ecosystem modeling. Species were classified into five PFTs (herb, shrub and liana, sclerophyll angiosperm tree, malacophyll angiosperm tree, gymnosperm), defined by a combination of phylogeny (angiosperm, gymnosperm), life form (tree, herb, shrub, liana), phenology (evergreen and deciduous) and leaf consistency (sclerophyll and malacophyll: sclerophyll leaves are hard, tough and stiffened; malacophyll leaves are soft).

2.2. Analytical models

2.2.1. Stomatal limitation

Medlyn et al. (2011) showed that the theory of optimal stomatal conductance leads, with some approximations, to a simple theoretical model that is closely analogous to widely used empirical models (Ball et al., 1987; Leuning, 1995; Arneth et al., 2002):

$$g_s \approx g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_a}, \quad (1)$$

Table 1

Parameter values across 22 species and 5 PFTs.

Species	PFT	No.	Literature source	b	g_1^*	V_{cmax}^*	S_f	Ψ_f
<i>Allocasuarina luehmannii</i>	Sclerophyll angiosperm tree	1	Posch and Bennett (2009)	1.08	1.99	50.7	2.12	−2.16
<i>Cinnamomum bodinieri</i>	Sclerophyll angiosperm tree	4	Liu et al. (2010)	−0.4	3.11	34.1	4.52	−1.52
<i>Eucalyptus pilularis</i>	Sclerophyll angiosperm tree	5	Kelly (unpublished)	0.94	2.22	69.38	1.82	−1.14
<i>Eucalyptus populnea</i>	Sclerophyll angiosperm tree	6	Kelly (unpublished)	0.15	3.15	52.94	2.06	−3.36
<i>Olea europaea</i> var. <i>Chemlali</i>	Sclerophyll angiosperm tree	14	Ennajeh et al. (2008)	1.28	2.72	125.83	0.4	−0.69
<i>Olea europaea</i> var. <i>Meski</i>	Sclerophyll angiosperm tree	15	Ennajeh et al. (2008)	0.49	3.16	72.52	0.51	−1.95
<i>Quercus coccifera</i>	Sclerophyll angiosperm tree	19	Peguero-Pina et al. (2009)	0.02	6.06	81.2	4.89	−1.66
<i>Quercus ilex</i>	Sclerophyll angiosperm tree	20	Peguero-Pina et al. (2009)	−0.11	6.12	75.62	0.73	−0.17
<i>Quercus suber</i>	Sclerophyll angiosperm tree	21	Peguero-Pina et al. (2009)	0.14	7.55	89.97	1.2	−3
<i>Broussonetia papyrifera</i>	Malacophyll angiosperm tree	2	Liu et al. (2010)	1.14	5.88	50.06	7.16	−1.32
<i>Platycarya longipes</i>	Malacophyll angiosperm tree	16	Liu et al. (2010)	0.66	5.85	37.16	2.53	−2.01
<i>Pteroceltis tatarinowii</i>	Malacophyll angiosperm tree	18	Liu et al. (2010)	−0.14	3.7	33.49	3.49	−1.79
<i>Ficus tikoua</i>	Shrub and liana	7	Liu et al. (2011)	0.07	6.17	42.78	3.34	−1.6
Medit. deciduous shrubs	Shrub and liana	11	Galmés et al. (2007)	0.49	4.82	61.87	3.89	−1.82
Medit. evergreen shrubs	Shrub and liana	12	Galmés et al. (2007)	0.68	4.72	27.81	2.02	−2
<i>Rosa cymosa</i>	Shrub and liana	22	Liu et al. (2010)	0.22	3.07	52.6	1.62	−2.35
<i>Glycine max</i>	Herb	8	Liu et al. (2005)	0.09	3.74	71	1.89	−1.37
<i>Glycine max</i>	Herb	9	Huang et al. (1975)	0.41	3.77	84.2	7.93	−0.65
<i>Helianthus annuus</i>	Herb	10	Tezara et al. (2008)	2.07	4.64	105.4	1.7	−1.03
Medit. herbs	Herb	13	Galmés et al. (2007), Medrano et al. (2009)	1.61	4.72	71.23	11.97	−0.99
<i>Cedrus atlantica</i>	Gymnosperm	3	Griew et al. (1988)	0.46	3.18	13.31	5.28	−2.31
<i>Pseudotsuga menziesii</i>	Gymnosperm	17	Griew et al. (1988)	0.79	3.13	13.16	–	–
PFT median values								
Sclerophyll angiosperm tree				0.15	3.15	72.52	1.82	−1.66
Malacophyll angiosperm tree				0.66	5.85	37.16	3.49	−1.79
Shrub and liana				0.35	4.77	47.69	2.68	−1.91
Herb				1.01	4.2	77.72	4.91	−1.01
Gymnosperm				0.63	3.16	13.23	5.28	−2.31

where D is the vapor pressure deficit at the leaf surface (kPa); C_a is the atmospheric CO_2 concentration at the leaf surface ($\mu mol\ mol^{-1}$); g_s is stomatal conductance to water vapor ($mol\ m^{-2}\ s^{-1}$), and g_0 is the leaf water vapor conductance when photosynthesis is zero ($mol\ m^{-2}\ s^{-1}$). The derivation of the model (Medlyn et al., 2011) provides an interpretation for the single model parameter g_1 ($kPa^{-0.5}$):

$$g_1 \propto \sqrt{\frac{\Gamma^*}{\lambda}}, \quad (2)$$

where Γ^* is the CO_2 compensation point in the absence of dark respiration ($\mu mol\ mol^{-1}$), and λ is the marginal water use efficiency ($\partial A / \partial E$, $mol\ C\ mol^{-1}\ H_2O$) (Medlyn et al., 2011). We estimated g_1 for each predawn leaf water potential from values of A and g_s by rearranging Eq. (1). The parameter g_0 is not part of the optimization. In the analysis, g_0 was estimated as the minimum value of g_s in each data set, similar to Manzoni et al. (2011) who set cuticular conductance to water vapor at 90% of the minimum measured g_s . The gas exchange data corresponding to the minimum measured g_s in each data set were excluded.

An exponential response curve of g_1 to Ψ_{pd} was fitted to each set of observations. Instead of setting the function shape responses in advance (e.g. Egea et al., 2011), we inferred them based on the data set. We attempted to fit the logistic function (cf. Tuzet et al., 2003), but the exponential function suggested by Mäkelä et al. (1996) fitted better:

$$g_1 = a \exp(b\Psi_{pd}), \quad (3)$$

where a and b are fitted parameters: a is the g_1 value at $\Psi_{pd} = 0$, and b represents the sensitivity of g_1 to Ψ_{pd} . Species adopting different water use strategies might be expected to differ in their estimated g_1 values under moist conditions (defined here as the value of g_1 when $\Psi_{pd} = -0.5$ MPa), and might also differ in their sensitivity to water stress as represented by b .

2.2.2. Non-stomatal limitation

The apparent effect of water stress on V_{cmax} was quantified from the measurements of A and g_s as follows. Firstly, we assumed that photosynthesis was Rubisco-limited under the conditions of the experiments. Note that it is alternatively possible to assume that photosynthesis was electron transport-limited, leading to an estimate of the apparent effect of water stress on J_{max} . Results (Fig. 4 in Appendix B) were closely similar, indicating that this method effectively estimates the effect of drought stress on the photosynthetic biochemistry. We estimated the maximum apparent V_{cmax} value when $\Psi_{pd} = 0$ (V_{cmax}^*). This estimate was obtained by inverting the expression for photosynthesis as follows:

$$V_{cmax} = \frac{(A + R_d)(C_i + K_m)}{(C_i - \Gamma^*)}, \quad (4)$$

where the value of C_i is obtained from measurements (Appendix A). We then estimated, for each data point, the photosynthesis (A_c) that would be obtained if drought stress only affected C_i , by calculating the photosynthetic rate obtained with maximum V_{cmax}^* and the observed C_i . The ratio of observed photosynthesis to the estimated photosynthesis (A/A_c) measures the apparent effect of water stress on V_{cmax} , as this ratio gives the decline in V_{cmax} that would be needed to obtain the observed photosynthetic rate.

The sensitivity of V_{cmax} to water availability was quantified using the logistic function (Tuzet et al., 2003):

$$f(\Psi_{pd}) = \frac{1 + \exp[S_f\Psi_f]}{1 + \exp[S_f(\Psi_f - \Psi_{pd})]}. \quad (5)$$

The function $f(\Psi_{pd})$ accounts for the relative effect of water stress on the apparent V_{cmax} . The form of this function allows a relatively flat response of apparent V_{cmax} under wet conditions, followed by a steeper decline, with a flattening again (toward zero) under the driest conditions. S_f is a sensitivity parameter indicating the steepness of the decline, while Ψ_f is a reference value indicating the water potential at which $f(\Psi_{pd})$ decreases to half of its maximum value. Species adopting different water use strategies might

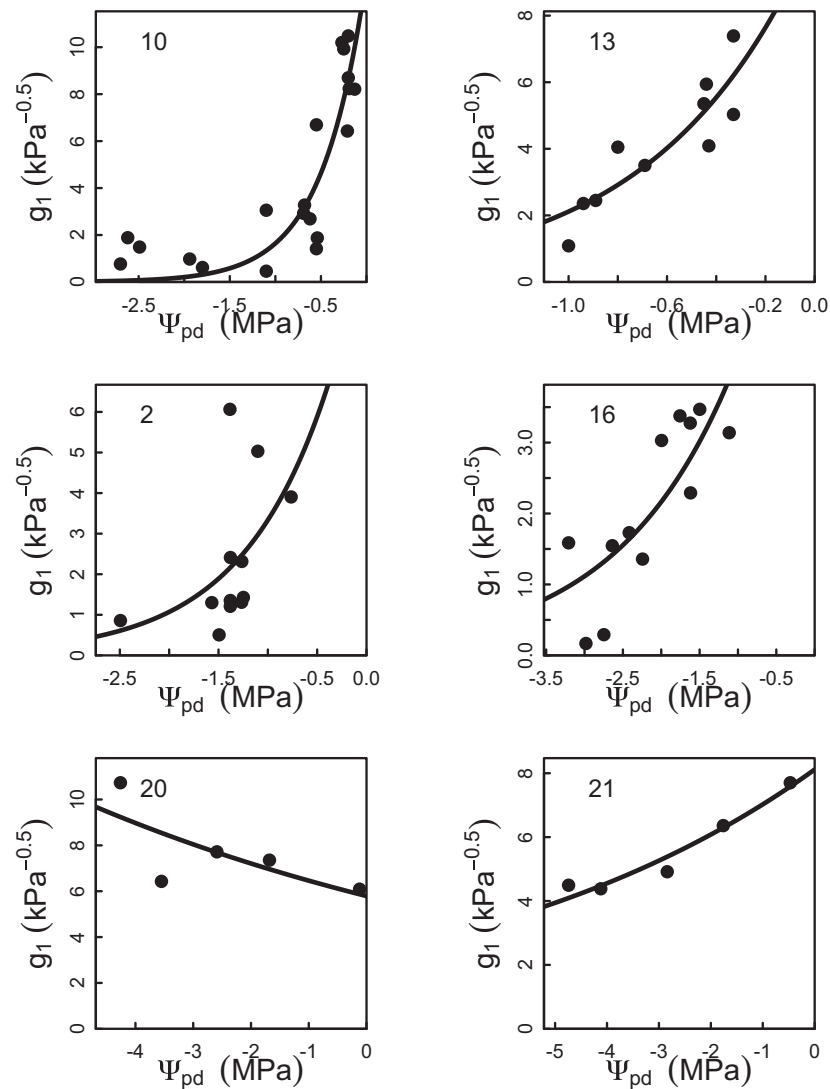


Fig. 3. Variability of g_1 responses to Ψ_{pd} from two data sets representative for each of three PFTs. Herbs: (10) *Helianthus annuus* and (13) *Mediterranean Herbs*; Malacophyll angiosperm tree: (2) *Broussonetia papyrifera* and (16) *Platycarya longipes*; Sclerophyll angiosperm tree: (20) *Quercus ilex* and (21) *Quercus suber*.

be expected to differ in their estimated V_{cmax} values under moist conditions, and might also differ in the sensitivity of apparent V_{cmax} to water stress (S_f) and reference water potential (Ψ_f).

There are two possible classes of causes for a reduction in apparent V_{cmax} : biochemical reductions in enzyme activity, or reductions in mesophyll conductance (g_m). With the data available, we are not able to distinguish between these two effects. However, we investigated the potential role of the two effects by calculating the implied drought response of one parameter when the other parameter was held constant. Firstly, we assumed g_m as constant at $0.15 \text{ mol m}^{-2} \text{ s}^{-1}$ (Niinemets et al., 2009a), and then estimated the decline of true V_{cmax} needed to account for observed photosynthesis decline with drought. Secondly, we assumed true V_{cmax} as unchanged from its fitted value at $\Psi_{pd} = 0$, and then estimated the decline in g_m needed to account for observed photosynthesis decline with drought.

2.3. Multivariate analysis

The fitted values of five parameter, S_f , Ψ_f , b , g_1^* (g_1 estimated at $\Psi_{pd} = -0.5 \text{ MPa}$) and V_{cmax}^* (apparent V_{cmax} estimated at $\Psi_{pd} = 0$), were entered as variables (traits) in a principal components analysis (PCA) based on their correlations across species, grouped

by PFTs. The results were interpreted in terms of emergent patterns of differences among PFTs. The analysis was written and run in R (R Development Core Team, 2010).

3. Results

3.1. Response of g_1 to water stress

Estimated parameter values from each data set, and median values for each PFT, are given in Table 1. Species differed greatly in their estimated g_1 values under moist conditions (parameter g_1^*), and the rates at which g_1 declined with water stress (parameter b). Differences in the response curves are shown in Fig. 3 (which shows selected species in detail; see also Fig. 2 in Appendix B) and Fig. 4 (which compares response curves for all species). Malacophylls generally (except *Pteroceltis tatarinowii*) had higher g_1^* values than sclerophylls (5.85 versus 3.15, based on PFT median values in Table 1; Fig. 3).

The rate of decline in g_1 with drought varied considerably among species, with g_1 remaining nearly constant in some species and declining severely with drought in others. For herbaceous species (except *Glycine max*), g_1 was found to decline more steeply with water stress than in trees. Shrubs and lianas showed intermediate

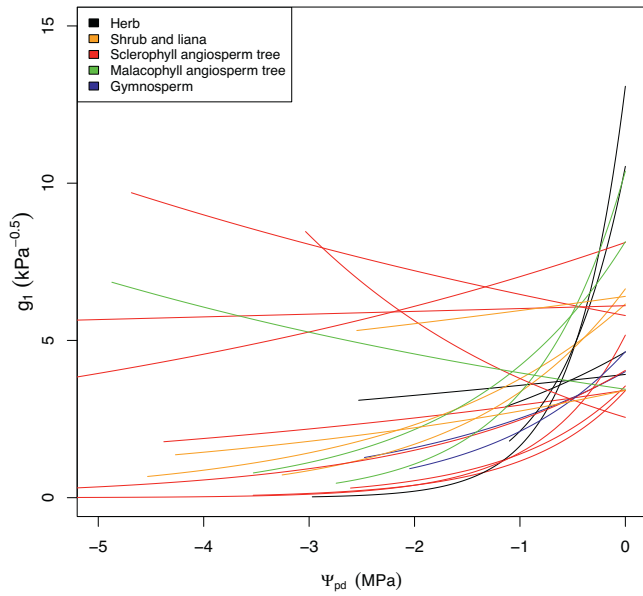


Fig. 4. g_1 responses to Ψ_{pd} from 22 data sets, grouped by PFT (herb, shrub and liana, sclerophyll angiosperm tree, malacophyll angiosperm tree, gymnosperm).

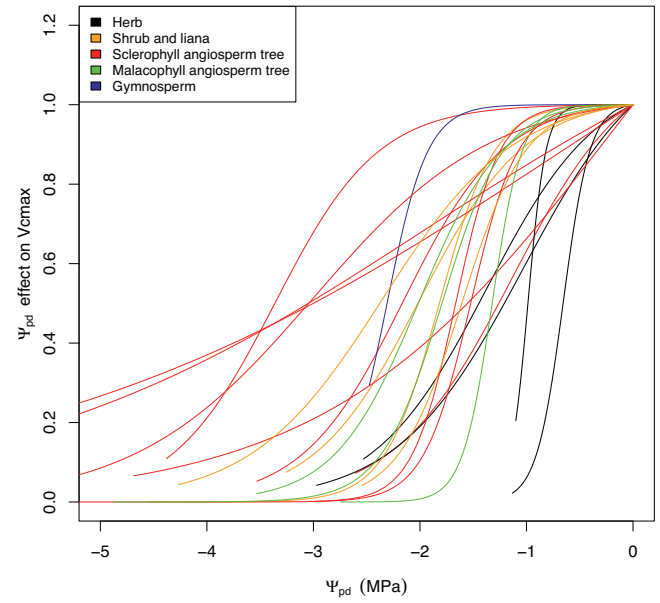


Fig. 6. V_{cmax} responses to Ψ_{pd} from 21 data sets, grouped by PFT (herb, shrub and liana, sclerophyll angiosperm tree, malacophyll angiosperm tree, gymnosperm).

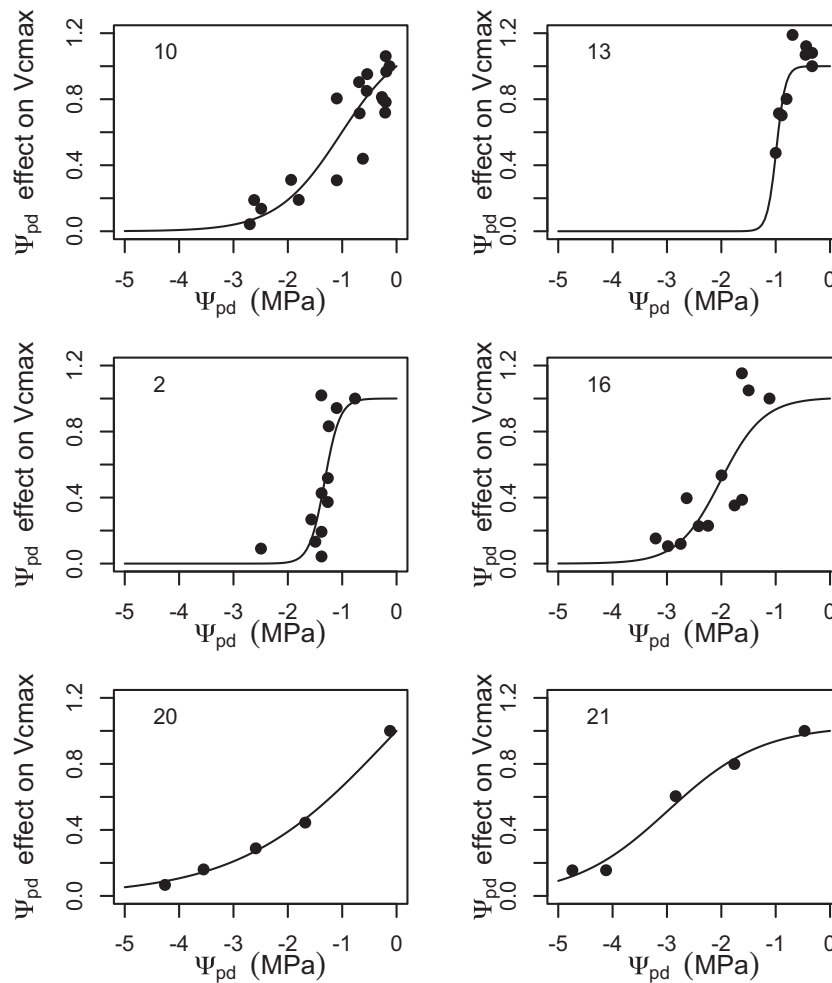


Fig. 5. Relative effect of Ψ_{pd} on V_{cmax} from two data sets representative for each of three PFTs. Herbs: (10) *Helianthus annuus* and (13) *Mediterranean Herbs*; Malacophyll angiosperm tree: (2) *Broussonetia papyrifera* and (16) *Platycarya longipes*; Sclerophyll angiosperm tree: (20) *Quercus ilex* and (21) *Quercus suber*.

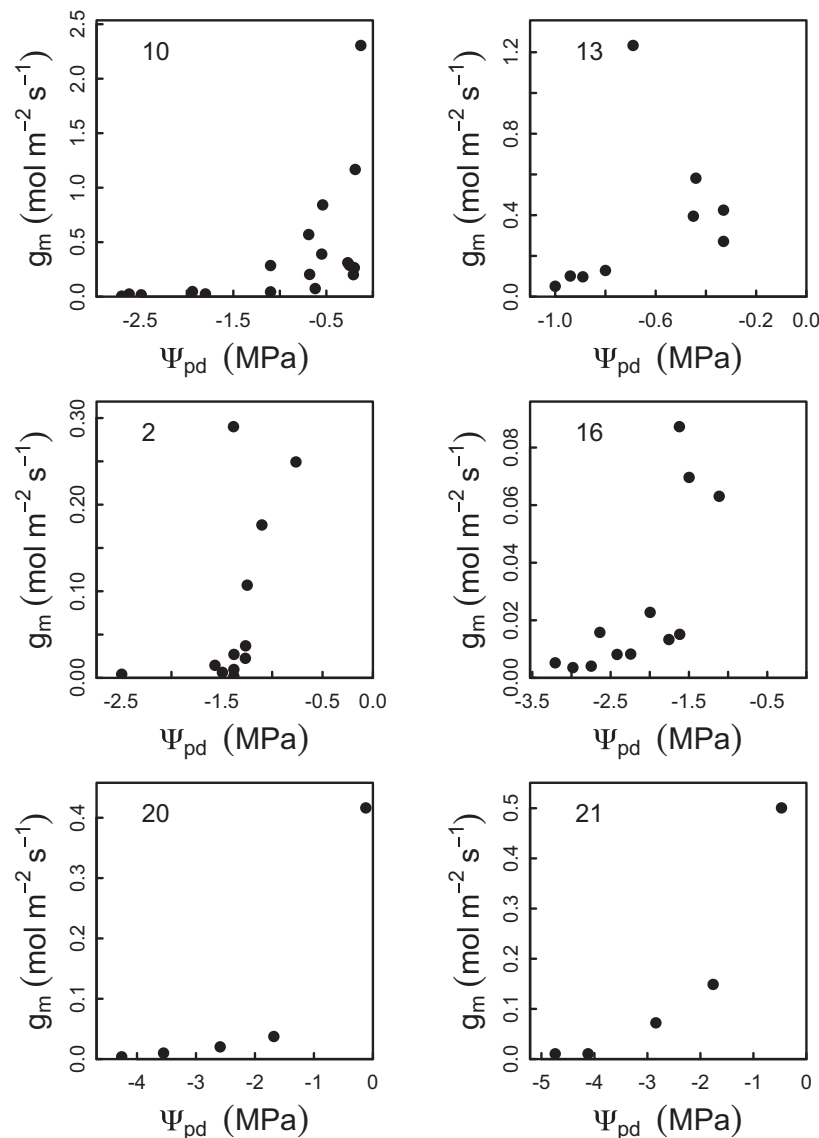


Fig. 7. Estimated responses of mesophyll conductance (g_m) to Ψ_{pd} under the assumption that true V_{cmax} is constant, for two data sets representative of each of three PFTs. These curves were estimated from data by assuming that true V_{cmax} is constant and equal to its observed value at $\Psi_{pd} = 0$, and calculating the reduction in g_m that would be needed to explain the observed reduction in apparent V_{cmax} .

responses. Woody species varied in their g_1 responses to drought. In the Mediterranean sclerophyll *Quercus* species (*Quercus ilex*, *Quercus suber*, *Quercus coccifera*) g_1 remained nearly constant or even increased with drought. The g_1 of *Cinnamomum bodinieri* increased with drought. The two *Eucalyptus* species (*Eucalyptus pilularis*, *Eucalyptus populnea*) had very different drought responses, according to their ecological niches: in the mesic tall-forest species *E. pilularis*, g_1 decreased rapidly with drought ($b = 0.94$) whereas in the semi-arid woodland species *E. populnea*, g_1 barely changed with drought ($b = 0.15$).

3.2. Response of apparent V_{cmax} to water stress

We quantified the non-stomatal limitation to photosynthesis by calculating Rubisco-limited photosynthesis (A_c) values for each species assuming that V_{cmax} is constant at the value shown under moist conditions. The ratio of measured A and estimated A_c then indicates the degree of non-stomatal limitation. The observations consistently show a decline in apparent V_{cmax} , and thus

a progressive increase in non-stomatal limitation, with increasing water stress (Fig. 5; see also Fig. 3 in Appendix B).

We explored the relative impact of drought-induced reductions on apparent V_{cmax} and g_1 on photosynthesis. The effect of water stress on measured photosynthesis was compared (1) with the effect on estimated photosynthesis when V_{cmax} was fixed (on the V_{cmax} value at the least negative Ψ_{pd} in each data set) and only g_1 changed, and (2) with the effect on estimated photosynthesis when g_1 was fixed and only V_{cmax} changed (see Fig. 5 in Appendix B). This analysis confirmed the strong inhibitory effect of water stress on apparent V_{cmax} .

All species showed a decline of apparent V_{cmax} as water availability declined, but species differed considerably in the parameters (Ψ_f and S_f) of this response. Different PFTs varied in the sensitivity to Ψ_{pd} . Trees had generally more negative Ψ_f than that of herbaceous species (Table 1), i.e. they showed the ability to continue active photosynthesis down to lower soil water potentials than herbaceous species. Among woody species, malacophylls showed higher V_{cmax} sensitivity (S_f) to drought than most sclerophylls (3.49 versus 1.82, based on PFT median values in Table 1; Fig. 5).

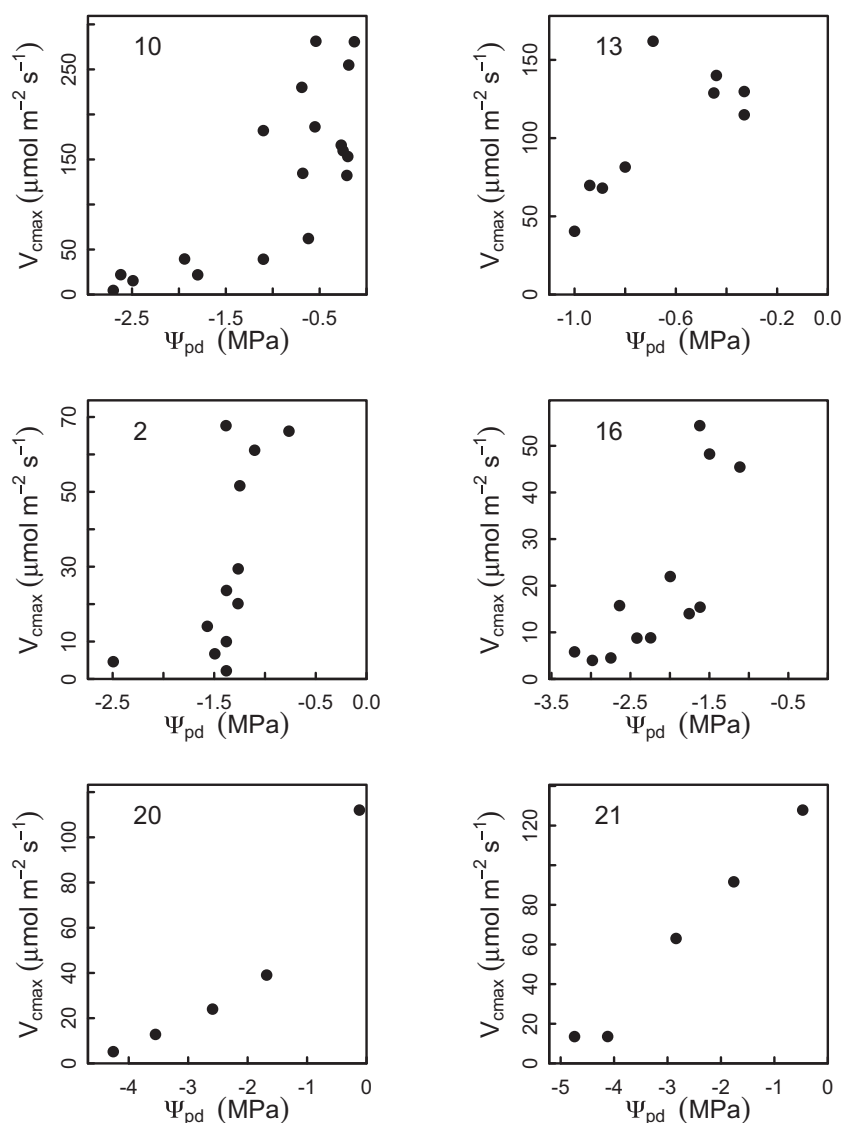


Fig. 8. Estimated responses of true V_{cmax} to Ψ_{pd} under the assumption that mesophyll conductance is constant, for two data sets representative of each of three PFTs. These curves were estimated from data by assuming that g_m is constant and equal to $0.15 \text{ mol m}^{-2} \text{ s}^{-1}$, and calculating the reduction in true V_{cmax} that would be needed to explain the observed reduction in apparent V_{cmax} .

Separate estimates of the response of either g_m (Fig. 7) or V_{cmax} (Fig. 8) to water stress show that both could equally well explain the declines in apparent V_{cmax} (Fig. 9).

3.3. Water relation strategies

PCA was conducted on 21 data sets that allowed estimation of all five parameters. *Pseudotsuga menziesii* was excluded because the $f(\Psi_{pd})$ curve could not be fitted, owing to the small number of data points.

The first principal component (PC1) shows the existence of one independent gradient, characterized by the positive correlation among V_{cmax}^* , b and Ψ_f (Fig. 9). It indicates that species with high V_{cmax}^* also tend to have a high sensitivity of both g_1 and V_{cmax} to drought. Species with this combination of traits are aligned to the right part of Fig. 9, with positive scores on the first principal component. The second principal component (PC2) shows the existence of another independent gradient, characterized by the positive correlation between g_1^* and S_f . Species with high values of g_1^* and S_f are aligned in the upper part of Fig. 9, with positive scores on the second principal component. The two principal components

together account for 58.7% of the total variation in these five parameters.

This analysis shows species scattered through a continuum, but nevertheless suggests some systematic patterns related to PFT membership. The first principal component (PC1) can be interpreted as a gradient in water relation strategies from woody to herbaceous species (explaining 35.2% of total variance). Compared with the herbaceous species, most of the woody species tended to have lower values of b (decreasing g_1 slowly to maintain more open stomata) and Ψ_f (maintaining high apparent V_{cmax}) down to lower water potential, and also lower V_{cmax} under wet conditions.

The second principal component (PC2) can be interpreted as a second gradient in water relation strategies from malacophyll to sclerophyll tree species (explaining 23.5% of total variance). Compared with malacophyll species (except *P. tatarinowii*, which has drought-adapted leaf anatomical structure and epidermal characteristics), sclerophyll species have lower values of g_1^* and S_f , corresponding to a combination of relatively low stomatal conductance under wet conditions, but with a slow decline of photosynthetic capacity under drought.

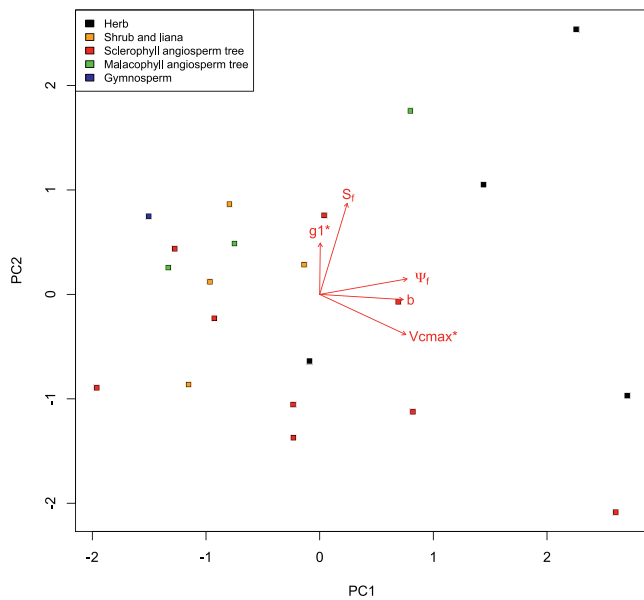


Fig. 9. Principal components analysis of the five traits, S_f , Ψ_i , b , g_1^* (g_1 estimated at $\Psi_{pd} = -0.5$ MPa) and V_{cmax}^* (apparent V_{cmax} estimated at $\Psi_{pd} = 0$). The first principal component (PC1) explained 35.2% of total variance, and the second principal component (PC2) explained 23.5% of total variance. The two principal components together accounted for 58.7% of the total variation in these five parameters.

4. Discussion

The goal of this analysis was to compare data sets in a modeling framework to identify key aspect for modeling drought effects on plant gas exchange. The results are consistent with other studies showing that both stomatal and non-stomatal processes are affected by drought (e.g. [Egea et al., 2011](#); [Keenan et al., 2010](#)). Our analysis shows that non-stomatal limitation is considerable and has in general a greater impact than that of stomatal limitation on photosynthetic rates. Photosynthesis under drought would be greatly overestimated if the decline in apparent V_{cmax} was not taken into account. Both assimilation rate and stomatal conductance decrease as pre-dawn leaf water potential declines, but assimilation rate usually decreases more – often many times more – than could be explained by a reduction in stomatal conductance (and g_1) alone (see Figs. 1 and 2 in Appendix B).

The analysis showed large variation among species and PFTs in drought effect on g_1 . This finding is consistent with those of [Manzoni et al. \(2011\)](#) who showed the shape of $\partial A/\partial E$ response curves to soil water availability varies according to PFT and climate. The near-constancy (or slight increase) of g_1 values with declining Ψ_{pd} , as shown in Mediterranean oaks, is consistent with their evident ability to thrive in a climate with a long summer dry season; this departs from Manzoni et al.'s (2011) generalization that $\partial A/\partial E$ increases monotonically with increasing water stress. Among the *Eucalyptus* species studied, *E. populnea* (from a xeric climate) and *E. pilularis* (from a mesic climate) notably differ in their g_1 responses (see Fig. 2 in Appendix B), consistent with [Manzoni et al.'s \(2011\)](#) indication of lower marginal WUE (i.e. higher g_1) in species from dry climates.

The large variation among PFTs in the drought effect on apparent V_{cmax} , as shown in our analysis, has not been reported before. It appears that species with high V_{cmax}^* under moist conditions also tend to be most sensitive to moisture stress, and that responses to moisture stress of g_1 and apparent V_{cmax} are correlated. The consistency between g_1^* and S_f (PC2 in [Fig. 6](#)) also suggests that water stress is associated with consistent limitations on g_1 and apparent V_{cmax} . However, the lack of correlation between V_{cmax}^* and

g_1^* indicates that photosynthetic capacity and water use strategy under moist conditions are not correlated. Our results also highlight the different strategies of drought response shown by species of different PFTs and from different hydro-climates.

Variation between species and PFTs in their responses to water deficit may relate to species-specific trade-offs between transpiration and vulnerability to hydraulic failure ([Berninger and Hari, 1993](#)). The Ψ_{pd} at which the g_s of sclerophyll trees (especially the Mediterranean oaks) approaches zero was often well below that of malacophyll trees, and was much lower than that of the herbs (see Fig. 1 in Appendix B). Sclerophyll trees are particularly noted for their larger turgor maintenance capacity (e.g. [Myers et al., 1997](#); [Prior et al., 1997](#)), and maintaining high stomatal conductance and photosynthetic capacity at low water potentials (e.g. [Turner, 1994](#); [Groom and Lamont, 1997](#); [Niinemets et al., 2009b](#)). Deciduous trees usually display greater stomatal sensitivity to increasing water deficit than semi-deciduous and evergreen sclerophyll trees ([Myers et al., 1997](#)). Malacophyll, even when “evergreen”, may have to shed their leaves to regulate water loss during prolonged drought ([Prior et al., 1997](#)).

The exponential decline of g_1 with decreasing soil water availability is consistent with the theoretical analysis on optimal stomatal response by [Mäkelä et al. \(1996\)](#). However, in their analysis, [Mäkelä et al. \(1996\)](#) assumed no drought effect on photosynthetic capacity. Future analysis of optimal stomatal responses to drought should consider how the reduction in apparent V_{cmax} with water limitation alters the optimal stomatal behavior. Our finding that apparent V_{cmax} is reduced is consistent with recent studies of mesophyll conductance (g_m) which report that g_m decreases with soil water deficit, and this effect may also contribute as much as the reduction in g_s to the decrease of A under water stress ([Flexas et al., 2012](#); [Keenan et al., 2010](#); [Perez-Martin et al., 2009](#)). The available data did not allow us to calculate the extent of g_m limitation on photosynthesis under water-stressed condition. However, declines in apparent V_{cmax} could be equally well explained by declines in either g_m ([Fig. 7](#)) or V_{cmax} ([Fig. 8](#)). Either way, the responses can be modeled adequately using the concept of apparent V_{cmax} .

The data available for this analysis were limited. Noise might have been introduced by digitizing figures in original publications, setting D values when not reported, and differences in experimental methods (especially given the large time span among the experiments). Although thousands of experiments have been done on plant response to drought, data sets containing enough information for model improvement are rare. Thus, the analysis highlights a need for further model-oriented experimental work to better define quantitative relationships between the several different aspects of drought response and other plant traits. Nevertheless, this study permits some strong conclusion for modeling: (1) it is necessary to represent non-stomatal limitation to carbon assimilation during drought, and (2) there are large differences among plant species in the values of key drought-response parameters, which appear to be related to PFT membership and climate. Without consideration of these two points, it is likely that models will underestimate short-term (within-season) response of primary production to drought while also underestimating the ability of drought-adapted taxa to maintain their function.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agrformet.2013.05.009>.

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