Drought limitations to leaf-level gas exchange: results from a model linking stomatal optimization and cohesion–tension theory

Kimberly A. Novick¹, Chelcy F. Miniat² & James M. Vose³

¹School of Public and Environmental Affairs, Indiana University, Bloomington, IN 47405, USA, ²USDA Forest Service, Coweeta Hydrologic Laboratory, Otto, NC 28734, USA and ³USDA Forest Service – Southern Research Station – Center for Integrated Forest Science, North Carolina State University, Department of Forestry and Environmental Resources, Raleigh, NC 27695-8008, USA

ABSTRACT

We merge concepts from stomatal optimization theory and cohesion–tension theory to examine the dynamics of three mechanisms that are potentially limiting to leaf-level gas exchange in trees during drought: (1) a ‘demand limitation’ driven by an assumption of optimal stomatal functioning; (2) ‘hydraulic limitation’ of water movement from the roots to the leaves; and (3) ‘non-stomatal’ limitations imposed by declining leaf water status within the leaf. Model results suggest that species-specific ‘economics’ of stomatal behaviour may play an important role in differentiating species along the continuum of isohydric to anisohydric behaviour; specifically, we show that non-stomatal and demand limitations may reduce stomatal conductance and increase leaf water potential, promoting wide safety margins characteristic of isohydric species. We used model results to develop a diagnostic framework to identify the most likely limiting mechanism to stomatal functioning during drought and showed that many of those features were commonly observed in field observations of tree water use dynamics. Direct comparisons of modelled and measured stomatal conductance further indicated that non-stomatal and demand limitations reproduced observed patterns of tree water use well for an isohydric species but that a hydraulic limitation likely applies in the case of an anisohydric species.

Key-words: stomatal conductance; transpiration; isohydric; anisohydric; water use efficiency; capacitance.

INTRODUCTION

The stomatal conductance to CO₂ (gₑ) is a critical determinant of plant response to drought (Oren et al. 1999; Sperry 2000; Flexas and Medrano 2002; McDowell et al. 2008) and directly influences photosynthetic assimilation (A), net primary productivity and mortality during periods of hydrologic stress (Leuning et al. 1995; Katul et al. 2000; McDowell et al. 2008; Katul et al. 2009; Medlyn et al. 2011). Achieving a mechanistic understanding of the dynamics of gₑ during drought – a long-studied topic (Jarvis 1976; Cowan and Farquhar 1977; Hari et al. 1986; Tyree and Sperry 1988) – has become especially important in light of forecasts for more frequent and/or severe drought events in coming decades (Huntington 2006; Cook et al. 2015).

Herein, we consider a number of different mechanisms by which drought-related perturbations in water availability and vapour pressure deficit may affect gₑ. Our study is focused in particular on the impacts of ‘meteorological droughts’ (Wilhite and Glantz 1985), which represent periods of precipitation deficiency, high temperatures and low humidity that can impact plant physiological functioning, even if they are relatively short in duration. The mechanisms that can limit gₑ during meteorological drought include (1) supply, or hydraulic, limitations to plant water uptake imposed by progressively decreasing soil water potential; (2) ‘demand limitations’ to gₑ imposed by excessively high vapour pressure deficit (hereafter D); and (3) non-stomatal limitations to a plant’s photosynthetic machinery imposed by declines in leaf water status.

In the following introductory sections, we will review the biophysical underpinnings of these limiting mechanisms and will also discuss the potentially mitigating role of hydraulic capacitance during drought periods. The presentation of a model framework that considers the coordinated interaction of hydraulic, demand and non-stomatal limitations during drought forms the foundation of the rest of the manuscript. The work is motivated by the recognition that improving our understanding of drought effects on gas exchange across species is necessary to reduce uncertainty in earth system models (Dietze et al. 2014) and to develop the management practices that can ultimately limit tree mortality and other deleterious drought effects (Grant et al. 2013).

Hydraulic limitations to gₑ

A growing body of literature (Meinzer et al. 2009; Choat et al. 2012; Martinez-Vilalta et al. 2014), grounded in cohesion–tension theory (van den Honert 1948; Tyree and Sperry 1988), highlights the important role of gₑ in preventing the development of excessively negative water potentials in the leaf or the xylem that may promote catastrophic cavitation. Because some plants have been found to operate more closely
to the point of hydraulic failure than others, plants are often classified along a continuum of leaf water potential regulation (Choat et al. 2012; Meinzer et al. 2014; Martínez-Vilalta et al. 2014). Plants that regulate \( g_c \) in such a way as to allow leaf water potential (hereafter \( \Psi_L \)) to approach the point at which excessive cavitation might occur are classified as more anisohydric. Examples of known anisohydric species include ring-porous Quercus species (Roman et al. 2015; Matheny et al. 2014) and Juniper species (Plaut et al. 2012; Meinzer et al. 2014). In contrast, isohydric plants regulate \( g_c \) to maintain a relatively stationary \( \Psi_L \) and a wide safety margin between the critical and actual water potentials (Choat et al. 2012; Sade et al. 2012; Klein et al. 2013) and include many Pinus species (Hacke et al. 2000; Plaut et al. 2012). The safety margin is often defined as the difference between midday water potential and the water potential at which a 50% loss in hydraulic conductivity occurs (\( \Psi_{50} \), Choat et al. 2012). However, another work suggests that the difference between midday leaf water potential and the water potential at which air begins to enter the xylem (\( \Psi_E \)) may be a more appropriate way to define the safety margin, at least during well-watered conditions (Meinzer et al. 2009).

**Demand limitations to \( g_c \)**

Another body of work has focused on applying concepts from stomatal optimization theory (Cowan 1986; Cowan and Farquhar 1977; Berninger and Hari 1993) to develop closed-form models for the relationship between \( g_c \) and the atmospheric demand for water vapour (Buckley 2005; Katul et al. 2009; Katul et al. 2010; Manzoni et al. 2011; Medlyn et al. 2011; Vico et al. 2011; Palmroth et al. 2013; Buckley and Schymanski 2014). These studies rely on the assumption that stomata function principally to maximize carbon uptake for a given water loss over some finite time interval, which leads to declines in \( g_c \) with increasing \( D \) during dry periods when atmospheric demand for water vapour is high. Thus, in this work, we refer to limitations imposed by the optimization constraint as demand limitations.

A decline in \( g_c \) with increasing \( D \) is often observed (Oren et al. 1999) and is an important component of empirical and theoretical models for \( g_c \) (Jarvis 1976; Leuning et al. 1995; Katul et al. 2000). The direct physiological mechanisms responsible for the response of \( g_c \) to \( D \) have been the subject of much debate (Schulze et al. 1972; Franks et al. 1997; Comstock and Mencuccini 1998; Buckley 2005). For the purpose of this study, these mechanisms do not need to be formally specified; rather, the optimization theory simply requires that they have evolved to allow plants to achieve optimal stomatal functioning (Berninger and Hari 1993; Buckley 2005). While this approach has long been recognized as a useful way to conceptualize stomatal dynamics (Cowan and Farquhar 1977; Hari et al. 1986; Mencuccini et al. 1996; Buckley 2005; Katul et al. 2009), with a few exceptions (e.g. Buckley 2005; Manzoni et al. 2013), the stomatal optimization approach has not been applied in a context that explicitly considers how hydraulic limitations to water supply in the leaf may also limit or co-limit gas exchange processes.

**Non-stomatal limitations to \( g_c \)**

Another research thrust has been focused on exploring how changes in leaf water status during periods of hydrologic stress affect gas exchange processes (Grassi and Magnani 2005; Niinemets et al. 2005; Lawlor and Tezara 2009) in ways that are independent, or at least quasi-independent, of stomatal limitations. These ‘non-stomatal limitations’ might include, for example, the generation of reactive oxygen species that damage ATP synthase (Tezara et al. 1999; Lawlor and Tezara 2009), other processes that limit the maximum carboxylation capacity of Rubisco (i.e. \( V_{C_{max}} \)) or diffusive limitations to the intercellular movement of CO2 imposed by declines in mesophyll conductance (Grassi and Magnani 2005; Niinemets et al. 2009; Flexas et al. 2012). A notable feature of the model presented here is the capacity for these non-stomatal limitations to drive reductions in \( g_c \), which is a feedback that has been proposed in other work (Buckley and Schymanski 2014).

**The mitigating role of hydraulic capacitance**

Finally, it is important to highlight recent advances in our understanding of how a reliance on stored water (or hydraulic capacitance) can mitigate limitations to \( g_c \). It is increasingly recognized that plants may rely on the gradual depletion of stored water pools to support gas exchange processes at midday, when light and also vapour pressure deficit are high (Zwiebel et al. 2001; Phillips et al. 2003; Scholz et al. 2011; Ward et al. 2013). Because hydraulic capacitance transiently decouples the flux of liquid water through the xylem from the transpiration flux, its role must be incorporated in any effort to link hydraulic and leaf-level limitations to gas exchange at sub-daily time steps.

A model framework that incorporates these concepts is developed and used here to address two principal research questions:

1 How do demand limitations, hydraulic limitations, non-stomatal limitations and hydraulic capacitance affect the dynamics of \( g_c \) during periods of hydrologic stress?

2 Can we detect and isolate these different limiting mechanisms in observed time series?

In developing the model, we draw on many previous studies that have already validated aspects of the hydraulic, demand and non-stomatal limitation mechanisms with observations (e.g. Berninger and Hari 1993; Grassi et al. 2009; Katul et al. 2010; Manzoni et al. 2011; Martínez-Vilalta et al. 2014). Here, we provide direct model-data comparisons for one isohydric and one anisohydric species and also explore the extent to which key model features are realized in observed time series of tree water uptake from a range of forest ecosystems in the Eastern USA. Our study is focused on general assessments of the model dynamics that we hope can motivate future hypothesis testing in empirical field studies or in studies that utilize publically accessible databases of plant physiological characteristics (e.g. the TRY database, Kattge et al. 2011).

**MODELLING CONSIDERATIONS**

As described in detail in the Supplementary Information (hereafter SI), the model development begins by linking Fick’s law

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of diffusion analogies for carbon assimilation ($A$) and transpiration ($E$) with a linear form of the Farquhar et al. (1980) model for photosynthesis. The model is not constrained by energy balance, which implies an infinite boundary layer conductance. The approach requires a third equation for $g_c$, which in this application comes from stomatal optimization theory. Models grounded in stomatal optimization theory begin with the assumption that stomata function explicitly to maximize the time and space integral of $A$ for a given $E$. It follows that the resulting leaf carbon gain function

$$f = A - \mu E$$

(1)

reaches a local maximum when $df/dg_c = 0$. The parameter $\mu$ relates to the sensitivity of $A$ to $E$ (i.e., $\partial A / \partial E$), which is assumed constant over short (daily) time intervals (Manzoni et al. 2013; Buckley and Schymanski 2014). The constant value at which $\partial A / \partial E$ operates over a given day is noted as $\lambda$ in some studies (Hari et al. 1986; Katul et al. 2009; Manzoni et al. 2011), although in other works, this same notation describes the variable’s inverse (i.e., $\partial E / \partial A$, Cowan and Farquhar 1977). Here, we adopt the notation $\mu$ to avoid confusion. An increase $\mu$ may be interpreted as an increase in the time-integrated carbon cost of transpired water over the timescales at which $\mu$ is presumed to be constant.

As discussed in detail in the SI, if $\mu$ is known or assumed, a formulation for $g_c$ may be achieved that does not require that the functional relationship between $g_c$ and $E$ be specified a priori. In the simple case of static leaf water potential ($\Psi_L$), the condition to maximize the function $f$ is given as (Cowan and Farquhar 1977; Hari et al. 1986; Katul et al. 2009)

$$\frac{df(g_c)}{dg_c} = \frac{\partial A}{c \partial g_c} - \mu \frac{\partial E}{c \partial g_c} = 0.$$  

(2)

As described in Katul et al. (2010), and presented in the SI, computing the derivatives ($\partial A / \partial g_c$) and ($\partial E / \partial g_c$) produces the following expression for $g_c$, for the case of static $\Psi_L$:

$$g_c = \left( \frac{a_1}{a_2 + sc_a} \right) \left[ -1 + \left( \frac{c_a}{a \mu D} \right)^{1/2} \right]$$

(static $\Psi_L$).  

(3)

Here, $a_1$ and $a_2$ are parameters relating to photosynthetic capacity, which depends on whether photosynthesis is light- or Rubisco-limited (e.g., $V_{C_{max}}$ and $J_{max}$ in other literature, refer to SI for details), $c_a$ is the atmospheric CO$_2$ concentration and $a$ is the ratio of diffusivity of H$_2$O to CO$_2$ in air equal to 1.6.

In the more realistic scenario of dynamic $\Psi_L$ at hourly to daily timescales, the dependency of $A$ on $\Psi_L$ and the sensitivity of $\Psi_L$ to $g_c$ must be considered. Specifically, the condition to maximize the function $f$, following Manzoni et al. (2011), becomes

$$\frac{df(g_c)}{dg_c} = \frac{\partial A}{c \partial g_c} + \frac{\partial A}{c \partial \Psi_L} \frac{\partial \Psi_L}{c \partial g_c} - \mu \frac{\partial E}{c \partial g_c} = 0$$

(dynamic $\Psi_L$).  

(4)

The dependency of $A$ on $\Psi_L$, which represents non-stomatal limitations to photosynthetic capacity, is expressed by relating $a_1$ to leaf water potential with an empirical function of the form

$$a_1 = a_{1,ww} \exp(b_1 (\Psi_L)^{b_2}).$$

(5)

after Vico & Porporato (2008), where $a_{1,ww}$ is the photosynthetic capacity under well-watered conditions and $b_1$ and $b_2$ are empirical constants. The relation between $g_c$ and $\Psi_L$ is developed by assuming that the transpiration rate, $E$, is equal to the sum of water flux through the stem ($f_l$) and the contribution of stored water to leaf water supply ($f_S$), giving $E = K(\Psi_L - \Psi_{L,0}) + f_S$ if gravitation head losses are neglected. This relation can be solved for $\Psi_L$, yielding

$$\Psi_L = \Psi_S - \frac{E - f_S}{K} = \Psi_S - \frac{(g_c + g_{c,o})f_l(T)D - f_S}{K}$$

(6)

where $\Psi_S$ is soil matric potential, $K$ is whole plant hydraulic conductance, $g_{c,o}$ is cuticular conductance to water vapour, $f_S$ is the capacitive flux of stored water and $f_l(T)$ is a temperature-dependent constant that is necessary to include when $D$ is expressed in units of kPa as opposed to a molar ratio.

The expression for $g_c$ that follows from Eqns. 4 to 6 is unwieldy and thus is not presented here, although model closure for $E, A, g_c$ and $\Psi_L$ is achieved provided $\mu$ is specified; more details on the implementation of the modelling approach are presented in SI.

It has been previously proposed that $\mu$ may increase during periods of water limitation (Makela et al. 1996), and recent work has confirmed the expectation of an adaptive $\mu$ by showing that across a wide range of species, $\mu$ increases with decreasing leaf water potential in a generic way (Manzoni et al. 2011). Here, we incorporate this adaptability of $\mu$ by using a modified version of the relationship proposed by Manzoni et al. (2011):

$$\mu = \mu_{WW} \exp\left( \beta_w \left( \Psi_{L,prev} + \Psi_o \right) \right)$$

for $\Psi_L < \Psi_o$

$$\mu = \mu_{WW}$$

or $\Psi_L \geq \Psi_o$

(7)

where $\mu_{WW}$ is the marginal water use efficiency for well-watered conditions, $\beta_w$ (which is negative) is a shape parameter and $\Psi_{L,prev}$ is the average midday $\Psi_L$ on the day preceding the simulation period. By linking $\mu$ to $\Psi_{L,prev}$ as opposed to $\Psi_L$, we effectively permit $\mu$ to vary over daily as opposed to hourly timescales, consistent with the expectation that it is relatively constant over the course of a given day. This formulation assumes that there is some range of $\Psi_L$ (i.e. $\Psi_o \leq \Psi_L \leq 0$) over which $\mu$ is insensitive to $\Psi_L$.

The optimality condition produces an atmospheric ‘demand’ limitation to stomatal conductance driven by increases in $D$ even in the absence of changes in soil water potential, which is evident in the dependence of $g_c$ on $D^{-1/2}$ in Eqn. 3. A ‘hydraulic’ limitation will apply when the $\Psi_L$ necessary to support the demand-limited $g_c$ is more negative than a prescribed critical minimum leaf water potential ($\Psi_{CRIT}$), below which rapid hydraulic failure may ensue. We note that it is not necessary to explicitly link $\Psi_{CRIT}$ to either $\Psi_o$ or $\Psi_{S0}$ for the purposes of exploring general model dynamics. When the $\Psi_L$ necessary to support the optimal

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$g_c$ is less than $\Psi_{\text{CRIT}}$, the formulation for $g_c$ is no longer derived from the optimization constraint; rather, the stomatal conductance is determined by inverting Eqn. 6 with $\Psi_L = \Psi_{\text{CRIT}}$.

To summarize, the model considers three potentially limiting mechanisms to $g_c$ and other gas exchange variables during drought and one mitigating factor. The first is the demand limitation to $g_c$, driven principally by $D$, which derives from the optimality assumption. The second is the hydraulic limitation imposed when the $\Psi_L$ necessary to sustain optimal $g_c$ is more negative than $\Psi_{\text{CRIT}}$; in this case, the formulation for $g_c$ is determined from Eqn. 6. The third is non-stomatal limitation to gas exchange, driven by the decrease in $a_1$ with increasing $\Psi_L$ (Eqn. 6) and/or the increase in $\mu$ with $\Psi_L$ (Eqn. 7). In addition, via the inclusion of a stored water flux term (i.e. $F_s$ in Eqn. 6), the model also accommodates the important role that hydraulic capacitance may play in mitigating any of these three limitations to gas exchange during periods of hydrologic stress.

**METHODS**

**Generalized model simulations – driving variables, parameterization and analysis**

All model simulations were driven by observations of $D$, photosynthetically active radiation ($Q$), air temperature and soil water potential ($\Psi_s$) that were observed within a 20-year-old loblolly pine forest near Durham, North Carolina from August 24 to September 12, 2005 (refer to SI and Stoy et al. 2006; Novick et al. 2014). During this time period, only 2 mm of precipitation fell on the site, promoting mild meteorological drought conditions characterized by increasing $D$ and steadily decreasing $\Psi_s$ (refer to supplementary Fig. S1).

The model was run for a range of scenarios reflecting various assumptions about the limiting mechanisms to leaf gas exchange and the role of stored stem water. First, the model was run for the simple case of no hydraulic constraint, no capacitance and no sensitivity of $\mu$ or $a_1$ to $\Psi_L$ (case 1, Table 1). In case 2, a hydraulic constraint is imposed by requiring $\Psi_{\text{CRIT}} > -1.8$ MPa, which is an arbitrary value selected to be higher than the lowest values of midday $\Psi_L$ observed for the case 1 simulations. In case 3, the parameter $\mu$ varies with $\Psi_L$ accordingly, with a sensitivity $\beta_\mu = -1.5$. In case 4, the parameter $a_1$ varies with $\Psi_L$, with a sensitivity $b_1 = -0.5$. Finally, case 5 is identical to case 2, but with an assumed contribution of stored water flux to $E$. The $F_s$ flux was assumed to be positive during the morning (representing depletion of stored water) and negative in the afternoon and evening (representing xylem refilling, refer to SI for more details), consistent with previously published $F_s$ data from a range of biomes (Phillips et al. 2003; Meinzer et al. 2004).

We focused our analysis of model outputs on the relationships between $E$, $g_c$ and $D$ over the course of the dry-down event. Of particular interest is the extent to which the relationship between $g_c$ and $D$ varies in time. In the case where the demand limitation is the only limitation to gas exchange, the relationship between these two variables takes the form

$$g_c = b + mD^{-1/2},$$

where the slope and intercept parameters may be predicted directly from the value of $c_g$, $a_1$, $a_2$ and $\mu$ (refer to Eqn. 3). When $\mu$ changes over the course of the dry-down event (reflecting non-stomatal limitations), or in the presence of a hydraulic limitation or hydraulic capacitance, the mathematical framework of the model does not permit elegant, analytical representations for the slope and intercept parameters. Thus, in this case, the parameters $b$ and $m$ were determined by directly linear regression of the modelled $g_c$ as a function of $D^{-1/2}$.

**Exploring the extent to which model features are observed in empirical data**

After summarizing the distinguishing features of each limiting mechanism to gas exchange, we explored the extent to which these features were observed in field observations of tree water use. In mixed canopies and in the absence of time-intensive measurements of leaf gas exchange, a convenient proxy for $E$ is xylem sap flux measurements (Oren et al. 1999). Thus, our application of the model framework focuses on the dynamics of the ‘apparent’ rates of transpiration and stomatal conductance observed in field sap flux data, hereafter $E_{\text{SF}}$ and $g_{c,\text{SF}}$, where the subscript ‘SF’ stands for sap flux. Practically, $E_{\text{SF}}$ is a proxy for $K(\Psi_s - \Psi_L)$, and $g_{c,\text{SF}}$ is thus

<table>
<thead>
<tr>
<th>Case</th>
<th>Sensitivity of $\mu$ to $\Psi_L$</th>
<th>Sensitivity of $a_1$ to $\Psi_L$</th>
<th>Hydraulic constraint</th>
<th>Capacitance?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (demand limitation only)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Case 2 (demand limitation + hydraulic limitation)</td>
<td>None</td>
<td>None</td>
<td>$\Psi_{\text{CRIT}} = -1.8$ MPa</td>
<td>No</td>
</tr>
<tr>
<td>Case 3 (demand limitation + non-stomatal limitation via $\mu$)</td>
<td>$\beta_\mu = -1.5$</td>
<td>None</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Case 4 (demand limitation + non-stomatal limitation via $V_{c,max}$)</td>
<td>None</td>
<td>$b_1 = -0.5$</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Case 5 (identical to case 2 but with a prescribed capacitive flux)</td>
<td>None</td>
<td>None</td>
<td>$\Psi_{\text{CRIT}} = -1.8$ MPa</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Other model parameters are specified in the SI.

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$g_{c, SF} = \frac{E_{SF}}{D_f(T)} = \frac{K(\Psi_T - \Psi_L)}{D_f(T)} - g_{c, o}.$  \hspace{1cm} (9)

In the event of little to no reliance on stored water and non-limiting boundary layer conductance, $E$ will be closely coupled to $E_{SF}$ and $g_c$ will be closely coupled to $g_{c, SF}$. Otherwise, predictive relationships determined for $E$ and $g_c$ may not apply to the proxies derived from sap flux data.

Xylem sap flux and relevant meteorological variables were monitored continuously from 2010 to 2012 in six Eastern US forest ecosystems as part of the USDA Forest Service Remote Assessment of Forest Ecosystem Stress (RAFES) project. Details on the sap flux methodology are presented in the SI. In each study site, we identified a period of 2–3 months during the growing season that included a significant dry-down period of at least 10 days and was associated with a clearly observable decline in soil moisture content. Data were then classified on the basis of soil moisture into relatively wet, intermediate or dry periods. The diurnal patterns of sap flux, the ratio of nocturnal to daytime sap flow and the slope and intercept of the function $g_{c, SF} = b + mD^{-1/2}$ were determined in each site period (refer to SI for details).

Species-specific model simulations for comparison with observations

To evaluate the ability of the model to reproduce observed gas exchange dynamics directly, we produced additional model simulations for two species (Pinus taeda and Quercus alba) growing in the Duke Upper RAFES site. We selected these species for two reasons. First, they span a gradient of isohydric to anisohydric behaviour. P. taeda, like many conifers, is known to be very isohydric (Hacke et al. 2000), whereas Q. alba is known to be more anisohydric (Roman et al. 2015). Second, the Duke Forest is an intensely studied ecosystem from which rich eco-physiological datasets have been produced (e.g. Hacke et al. 2000; Maherali et al. 2006; Domec et al. 2010), facilitating site- and species-specific model parameterizations. Details on the parameterization approach are given in the SI. The biggest difference between the two parameterizations was introduced through the formulation for $K$. Using hydraulic vulnerability curves previously reported by Maherali et al. (2006), $K$ was prescribed to be relatively low and insensitive to $\Psi_L$ for the isohydric P. taeda and relatively high and highly sensitive to $\Psi_L$ for the anisohydric Q. alba.

RESULTS

The effect of the various limitations on the dynamics of $g_c$, $E$ and $\Psi_L$

In all model simulations, the daytime $g_c$ decreased as the dry down progressed (Fig. 1a–c), reflecting demand limitation as $D$ increased. When the optimization constraint is the only limitation to gas exchange (i.e. case 1), the decline in daytime $g_c$ did not translate into a decline in daytime $E$ (Fig. 1d–f, black lines) because a higher $D$ will drive $E$ forward even as it reduces $g_c$ (refer to Eqn. S2 of the SI). A hydraulic limitation to gas exchange reduced the magnitude of daytime $g_c$ and $E$ relative to case 1 (black dashed line in Fig. 1a–f). When hydraulic limitations apply, they may be distinguished by the relatively constant $E$ during the course of the day, over which timescales $\Psi_S$ does not change considerably (Fig. 1e,f). Non-stomatal limitations to gas exchange, imposed either through increasing $\mu$ or decreasing $a_1$, also reduced the magnitude of both $g_c$ and $E$ relative to case 1 (gray lines in Fig. 1a–f). These reductions reflect the fact that metabolic limitations to photosynthetic functioning feedback into declines in $g_c$ via the optimization constraint.

An important consequence of reductions to $g_c$ imposed by non-stomatal or hydraulic limitations is an increase in the $\Psi_L$ necessary to support the optimized gas exchange (Fig. 1g–h). In fact, for the scenarios presented in Fig. 1, minimum midday $\Psi_L$ near the end of the dry-down period was similar to that at the start of the dry-down period (\(-1.5\) to \(-1.8\) MPa) regardless of whether hydraulic or non-stomatal limitations were applied, even though no explicit hydraulic constraint was imposed in the latter case. In the absence of either a non-stomatal or hydraulic limitation, the $\Psi_L$ necessary to support the optimized gas exchange becomes excessively low near the end of the dry-down event (<\(-6\) MPa, Fig. 1i), with large associated declines in $K$ (Fig. 1i).

The relationship between $g_c$ and $D$ for the various model scenarios

When the optimization constraint is the only limitation to gas exchange, the slope of the relation between $g_c$ and $D$ (i.e. $m$) during conditions of non-limiting light is stationary over the course of the drought event (Fig. 2a). Non-stomatal limitations tend to decrease both $g_c$ and the magnitude of $m$ monotonically over the course of the dry-down event (Fig. 2e–h). This is true regardless of the mechanism by which they are imposed (i.e. variable $\mu$ or variable $a_1$), although the rate of change of $m$ depends on the sensitivity parameters $b_1$ and $\beta_s$ (Fig. 3a,b, respectively). The effect of hydraulic limitation on the relationship between $g_c$ and $D$ depends on the value of $\Psi_{CRIT}$. When $\Psi_{CRIT}$ is relatively low, the slope parameter $m$ increases over the course of the dry-down event (Figs. 2d & 3c). However, for higher $\Psi_{CRIT}$, the slope parameter $m$ may decrease in time as the difference between $\Psi_{CRIT}$ and $\Psi_S$ becomes smaller. In the extreme case where $\Psi_{CRIT} > \Psi_S$, gas exchange no longer proceeds, and the slope parameter $m$ is driven to zero (Fig. 3c).

An interesting feature of the model simulations is that the hysteresis in the relationship between hourly $E$ and $D$, when visualized over the course of a single day, tends to increase as the dry-down progresses (Fig. 4a–c). This feature is most pronounced when the optimization constraint is the only mechanism limiting gas exchange (Fig. 4a–c, black lines) and is driven by a phase lag between radiation (which peaks around noon) and $D$ (which peaks several hours later). Another interesting feature of the demand limitation is the capacity for $E$ to peak at some intermediate $D$ provided $\mu$ is sufficiently high (Fig. 4d).
Mediating influence of stored water under hydraulic limitation

A defining characteristic of a reliance on stored water is a decoupling between $E$ and the stem water flux (e.g. $E_{SF}$), with the contribution of the stored water flux to $E_{SF}$ being relatively low during daytime periods and relatively high during afternoon and nocturnal periods (Fig. 5a–b). The dynamics of leaf-level $g_c$ and the stomatal conductance that can be inferred from stem sap flux (e.g. $g_{c, SF}$) are also decoupled in the case of a strong reliance on stored water, with $E_{SF}$ and $g_{c, SF}$ underestimating the true $E$ and $g_c$ in the morning and overestimating them in the afternoon (Fig. 5a,b). Additionally, as illustrated in Fig. 5a, the $F_s$ assumed in these simulations requires some evening refilling of depleted water stores, leading to relatively high $E_{SF}$ during the hours between sunset and midnight.

The defining features of the limiting mechanisms to gas exchange and their links to field observations

The defining characteristics of demand, hydraulic and non-stomatal limitations are summarized in Table 2. It is important to note that these features apply to the case of little to no reliance on stored water. If hydraulic capacitance is large, then transpiration inferred from stem sap flux (e.g. $E_{SF}$) and $E$ become decoupled (as do $g_c$ and $g_{c, SF}$, Fig. 5). The occurrence of nocturnal stem water movement in order to refill stored water pools and embolized xylem elements may be an important indicator of a reliance on hydraulic capacitance (Fig. 5a).

Many of the distinguishing features of each limitation scenario were realized in the sap flux observations, as detailed in Table 3. Even during relatively well-watered conditions, the $g_{c, SF}$ decreased significantly with increasing $D$ according
to \( g_{c, SF} = b + mD^{-1/2} \) for nearly every site species, which is an evidence of the demand limitation. A trend of increasing clockwise hysteresis in the diurnal relationships between \( E \) and \( g_c \), another hallmark of the demand limitation, was observed in 7 of the 12 site species (Table 3). A graphical presentation of these dynamics is included as SI Fig. S3.

Stationary midday \( E_{SF} \), which may be indicative of a hydraulic limitation, was observed for four of the site species combinations: Liriodendron tulipifera and \( P. taeda \) in the Duke Lower site, \( P. palustris \) in the Jones Center site and \( P. taeda \) in the Crossett EF site, noting that the latter two sites are the most xeric sites in the network. In a number of other sites, the relationship between \( E_{SF} \) and \( D \) was peaked, with a clear maximum \( E_{SF} \) occurring at an intermediate \( D \). Such a feature may evolve from the demand constraint (Katul et al. 2010; Manzoni et al. 2011) if \( \mu \) is sufficiently high (Fig. 4d).

The slope parameter \( m \) decreased as conditions progressed from wet to dry in most site species, which is indicative of non-stomatal limitation in the absence of stations midday \( E_{SF} \). In the Jones Center’s \( P. palustris \) and in the Crossett EF site’s \( P. taeda \) trees, the ratio of nocturnal:total \( E_{SF} \) increased significantly over the course of the dry-down event, to a ratio of 0.21 in the former and 0.34 in the latter. Thus, in these sites, a reliance on hydraulic capacitance may alleviate the hydraulic or non-stomatal limitations to gas exchange processes.

### Evaluating the model's ability to reproduce observed water use dynamics for an isohydric and anisohydric species

The results presented in Table 3 show that many of the features of the model are realized in the observed time series. For a more formal test of the functionality of the model, we compare measured and modelled \( g_c \) for two species growing in the Duke Upper site: the isohydric \( P. taeda \) and anisohydric \( Q. alba \). We focus our evaluation on daily averaged midday \( g_c \) during periods of high-light conditions, when boundary layer conductance is likely to be high (consistent with model assumptions). The conductance data were normalized by their well-watered values to obviate the need to scale the leaf-level model results to match the tree-level sap flux data. Finally, because the results of non-stomatal limitations on gas exchange are qualitatively similar regardless of whether they are imposed by variable \( \mu \) or \( a_1 \) (i.e. Figs. 2 & 3), this comparison is simplified by considering only non-stomatal limitations imposed by variable \( \mu \).

For \( P. taeda \), the model reproduced well the change in normalized midday \( g_c \) (Fig. 6a). Regardless of the choice of \( \beta_o \), the correlation coefficient exceeds 0.80, and the mean absolute error was less than 10%. However, the lowest bias error was associated with an intermediate sensitivity of \( \mu \) to \( \Psi_L \) (\( \beta_o = -0.56 \), filled circles in Fig. 6a). It is important to note that
error was higher (12–17%) for the simulations illustrated in Fig. 6c. These scenarios represent a non-stomatal limitation with a high $\beta_o$ (1.5) and non-stomatal + hydraulic limitations with $\Psi_{CRIT} = -0.55$ or $-1.1$ MPa. We note that $-0.55$ and $-1.1$ MPa are representative of the $\Psi_e$ and $\Psi_{50}$, respectively, previously determined for this site species (Maherali et al. 2006). When no hydraulic limitation applies (triangles in Fig. 6c), the modelled $g_c$ is driven to zero late in the drawdown by hydraulic failure (i.e. $\Psi_L$ becomes excessively low and $K$ is driven to zero). When the $\Psi_{CRIT} = -0.55$ MPa (crosses in Fig. 6c), the modelled $g_c$ is also driven to zero late in the drawdown when the $\Psi_S$ becomes more negative than $\Psi_{CRIT}$. When $\Psi_{CRIT} = -1.1$ MPa and $\beta_o = 1.5$ (circles in Fig. 6c), the modelled $g_c$ remains positive throughout the simulation period but is nonetheless underestimated late in the drawdown when the measured $g_c$ is low. This model-data mismatch could be explained by capacitance, which is implied by the fact that the ratio of nocturnal:total daily $E_{SF}$ increased for this species late in the dry-down period (Table 3). It could also be explained by the fact that Q. alba trees tend to have deep roots, which would increase the integrated $\Psi_L$ over the rooting depth (Roman et al. 2015). Finally, the underestimation could be linked to an imperfect parameterization scheme that could be improved with additional benchmarking. For the purposes of this particular study, however, the most relevant result is that a hydraulic limitation likely applies for this site species.

**DISCUSSION**

The model framework presented here was designed to explore how the dynamics of gas exchange rates are limited or co-limited by demand limitations linked to optimal stomatal functioning, hydraulic constraints and non-stomatal limitations to leaf physiological functioning. We used the model outputs to illustrate that gas exchange dynamics for each limitation are associated with unique dynamical characteristics (Table 3) and confirmed that these features are often realized in empirical field observations of tree water use dynamics (i.e. Table 3, Fig. 6). A direct model-data comparison also confirmed the functionality of the model (i.e. Fig. 6).

We showed, using both model outputs and empirical observations, that demand and non-stomatal limitations to gas exchange can sufficiently reduce the demand for water in the leaf such that direct hydraulic limitations can often be avoided (i.e. Figs. 1 & 6). Specifically, there is a tendency for $g_c$ to decrease rapidly with increasing $D$ when $\mu$ increased, or $a_1$ decreased, with declining leaf water status (Fig. 2). As a result, excessive declines in $\Psi_L$ are avoided, promoting larger hydraulic safety margins. When viewed in light of recent synthesis studies showing that, when compared with angiosperms, gymnosperms tend to have higher $\mu$ (Manzoni et al. 2011) and also higher safety margins (Choat et al. 2012; Johnson et al. 2012), the results from the present study highlight that species-specific ‘economics’ of stomatal behaviour (as represented by the magnitude of $\mu$) may play an important role in differentiating species along the continuum of isohydric to anisohydric behaviour. This implication will be discussed in more detail in the
Drought limitations to gas exchange

The trajectory of hourly leaf-specific transpiration ($E$) as a function of $D$ during different stages of the dry down (a–c). When the demand limitation imposed by the optimization constraint is the only limiting mechanism to gas exchange (black lines), increasing clockwise hysteresis as the drought progresses is observed. Both non-stomatal (gray lines) and hydraulic limitations (black dashed lines) tend to reduce the extent of the hysteresis and the magnitude of the gas exchange variables. The direction of the hysteresis, which is the same in all panels, is indicated in (b). Panel (d) shows that when the parameter $\mu$ is high (in this case $80 \, \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), the demand limitation produces a peak in daytime $E$ at intermediate $D$. The value of $\mu$ associated with the case 1 simulations shown in (a)–(c) was $21 \, \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Finally, we note that the demand limitation can also promote the occurrence of a maximum $E$ at some intermediate value of $D$ (Fig. 5d), which cannot be produced by hydraulic limitations alone unless $K$ declines rapidly over the course of a given day. A peaked relationship between $E_{SF}$ and $D$ was observed in many of the empirical time series (Table 3).

The relationship between the degree of isohydry and the limiting mechanisms to gas exchange

Our study is novel in that our model proposes that non-stomatal limitations to gas exchange feedback through the optimization constraint to reduce $g_c$ and increase $\Psi_L$ (Fig. 2) and thus may be an important determinant of a plant’s positioning along the isohydric–anisohydric spectrum. In support of this proposition, we show that characteristics of a demand and non-stomatal limitations to gas exchange (i.e. significant relationship between $g_c$ and $D^{-1/2}$, increasing hysteresis in the relationship between $E$ and $D$, decreasing $m$ under dryer conditions) were realized in empirical observations of tree water use from a range of species growing in a range of Eastern US biomes (Table 3).

We also demonstrated, through a species-specific calibration of the model, that demand and non-stomatal limitations to gas exchange together explained well the dynamics of stomatal conductance observed for *P. taeda*, which is an isohydric species (Fig. 6a). In particular, these limitations promoted relatively stationary midday $\Psi_L$ (Fig. 6b) that remained greater or higher than both the $\Psi_e$ and $\Psi_{50}$ previously reported for this site species (Maherali et al. 2006). In the case of the more anisohydric *Q. alba*, we showed that a hydraulic constraint was necessary, in addition to non-stomatal and demand limitations, to prevent $\Psi_L$ from dropping below the species-specific $\Psi_{L,50}$, at which point the model predicts that hydraulic failure rapidly ensues (Fig. 6c–d). Even when a hydraulic constraint is applied, the

**Succeeding texts,** following a brief discussion on some of the features of the demand limitation to gas exchange imposed by the optimization constraint.

**Some notable characteristics of the demand limitation**

The demand limitation to $g_c$ is driven by the assumption that stomatal behaviour has evolved to achieve the goal of maximizing carbon uptake while minimizing water loss, which requires a decrease in $g_c$ with increasing $D$ (Katul et al. 2009; Figs. 1, 2). An important characteristic of the optimization constraint is a strong relationship between $g_c$ and $D^{-1/2}$ (Fig. 2); in the absence of non-stomatal or hydraulic limitations to gas exchange, the parameters of this relationship do not change as drought progresses (i.e. Fig. 2a). A significant relationship between $g_c$ and $D^{-1/2}$ was detected in most of the empirical time series (Table 3). Furthermore, previous work has shown that the relationship between $g_c$ and $D^{-1/2}$ that evolves from the optimization constraint is consistent with a wealth of previously published results (Oren et al. 1999; Katul et al. 2009).

When the full diurnal range of light conditions is considered, the demand constraint produces pronounced clockwise hysteresis in the relationship between gas exchange variables and $D$ (Figs. 2 & 3), which increases as a dry-down event progresses in the absence of significant non-stomatal or hydraulic limitation (Figs. 2 & 3). Similar clockwise hysteresis has been observed in other studies (Wullschleger et al. 1998; O’Grady et al. 1999; Ford et al. 2004; Ewers et al. 2005; Zhang et al. 2014), although again, the mechanism promoting this hysteresis is not clear (O’Grady et al. 1999; Unsworth et al. 2004; Zhang et al. 2014). Our results suggest that the principle cause of this hysteresis is the phase lag between light (i.e. $Q$) and $D$, confirming results from Zhang et al. (2014) and Ford et al. (2005).

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model still underestimates the stomatal conductance during the driest periods. This underestimation may be linked to hydraulic capacitance and species-specific differences in rooting depth or may reflect an imperfect parameterization scheme that could be improved upon with further model benchmarking.

The literature provides ample support for the notion that non-stomatal constraints, including reductions in photosynthetic capacity (i.e. $V_{cmax}$) and mesophyll conductance, are important controls on plant gas exchange during drought. For example, many studies report that $V_{cmax}$ declines with declining soil water potential, often by orders of magnitude (Flexas et al. 2002; Limousin et al. 2010; Zhou et al. 2014) and that these limitations combined can explain 30–50% of the observed declines in photosynthesis during drought periods (Limousin et al. 2010; Zhou et al. 2014). To our knowledge, no previously published study reports on the time evolution of $V_{cmax}$, $g_c$, $\Psi_L$ and the hydraulic safety margin during drought for multiple species spanning a gradient of isohydric and anisohydric behaviour. Such a dataset is certainly motivated by this study, as it would present a unique opportunity for a more rigorous evaluation of model predictions. Nonetheless, previous work reporting on a direct relationship between mesophyll conductance and $g_c$, both within and across species (Limousin et al. 2010; Flexas et al. 2012; Niinemets et al. 2005; Loreto et al. 1992), provides some empirical evidence that a feedback between mesophyll conductance and $g_c$ driven by the optimization constraint is possible, even if those relationships are insufficient to confirm the existence of this process directly.

### Utility and limitations of the model and diagnostic framework

In developing the model, we strove to find a balance between analytical tractability and physical realism, which necessitated the inclusions of a number of simplifying assumptions into the model framework. First, our model is based on a linearized variant of the Farquhar et al. (1980) photosynthesis model, which may bias the model if it is applied to conditions characterized by high $c_a$ (Katul et al. 2010). Second, in order to maintain analytical tractability, the model also neglects the effect of dynamic temperature on optimal gas exchange, although recent work suggests that those dynamics may be important (Duursma et al. 2014). Finally, our model also assumes that boundary layer conductance is never limiting, which may be a reasonable assumption during most daytime periods (Kim et al. 2014) but may not be a good assumption during early morning or when humidity is high. Our formulation of the stored water flux is rather coarse and requires that it be specified; future developments could focus on more directly integrating the role of capacitance in the optimization framework. We also note that modelled processes discussed here are representative of a rather specific combination of physiological capacity.

### Table 2. The salient identifying characteristics of demand, hydraulic and non-stomatal limitations to gas exchange, which may inform the diagnosis of limiting mechanisms in field observations

<table>
<thead>
<tr>
<th>Feature</th>
<th>Demand limitation</th>
<th>Hydraulic limitation</th>
<th>Non-stomatal limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hysteresis between $E$ and $D$ at hourly timescales</td>
<td>Increases as drought progresses</td>
<td>Reduced compared with the case of demand limitation alone</td>
<td>Reduced compared with the case of demand limitation alone</td>
</tr>
<tr>
<td>Hysteresis between midday $g_c$ and $D$ at daily timescales</td>
<td>None</td>
<td>Pronounced</td>
<td>Pronounced</td>
</tr>
<tr>
<td>Relationship between $g_c$ and $D^{-1/2}$</td>
<td>Does not change as drought progresses</td>
<td>The slope parameter $m$ may increase or decrease</td>
<td>The slope parameter $m$ decreases over the course of the drought</td>
</tr>
<tr>
<td>Other features</td>
<td>Potential for $E$ to peak at some intermediate $D$</td>
<td>Stationary $E$ and $\Psi_L$ during midday</td>
<td>Promotes less negative $\Psi_L$ during periods of hydrologic stress</td>
</tr>
</tbody>
</table>

Figure 5. A reliance on stored water decouples the dynamics of $E$ and $E_{SF}$ (a) and $g_c$ and $g_{c, SF}$ (b).
Table 3. Characteristics of the study sites and features of the observed dynamics of stem water flow ($E_{sf}$) and the apparent stomatal conductance derivable from $E_{sf}$ (i.e. $g_{c,sf}$; refer to Eqn. 9) for relatively dry, intermediate (Int) and wet conditions.

<table>
<thead>
<tr>
<th>Site</th>
<th>Lat (N) Lon (W)</th>
<th>Species</th>
<th>$m (r^2)$</th>
<th>$\Delta m$</th>
<th>Ratio of nocturnal to total daily $E_{sf}$</th>
<th>Patterns in daytime $E$</th>
<th>Hyst?</th>
<th>Governing mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wet</td>
<td>Int</td>
<td>Dry</td>
<td>Wet</td>
<td>Int</td>
<td>Dry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dry-wet</td>
<td>Int-wet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coweeta</td>
<td>33.05 83.42</td>
<td><em>Quercus alba</em></td>
<td>8 (.48)</td>
<td>5 (.18)</td>
<td>6 (.38)</td>
<td>.12</td>
<td>.11</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acer rubrum</em></td>
<td>2 (0.9)</td>
<td>ns</td>
<td>ns</td>
<td>.16</td>
<td>.12</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Liriodendron tulipifera</em></td>
<td>3 (.43)</td>
<td>1 (.29)</td>
<td>ns</td>
<td>.17</td>
<td>.11</td>
<td>.15</td>
</tr>
<tr>
<td>Duke Lower</td>
<td>33.987909</td>
<td><em>Acer rubrum</em></td>
<td>28 (.93)</td>
<td>22 (.91)</td>
<td>22 (.91)</td>
<td>.08</td>
<td>.07</td>
<td>.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pinus taeda</em></td>
<td>10 (.63)</td>
<td>6 (.63)</td>
<td>6 (.50)</td>
<td>.12</td>
<td>.09</td>
<td>.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Liriodendron tulipifera</em></td>
<td>12 (.71)</td>
<td>10 (.86)</td>
<td>9 (.86)</td>
<td>.09</td>
<td>.07</td>
<td>.09</td>
</tr>
<tr>
<td>Duke Upper</td>
<td>33.987909</td>
<td><em>Acer rubrum</em></td>
<td>23 (.89)</td>
<td>19 (.82)</td>
<td>14 (.56)</td>
<td>.04</td>
<td>.04</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pinus taeda</em></td>
<td>16 (.84)</td>
<td>15 (.85)</td>
<td>11 (.36)</td>
<td>.13</td>
<td>.12</td>
<td>.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Quercus alba</em></td>
<td>19 (.80)</td>
<td>22 (.71)</td>
<td>21 (.58)</td>
<td>.1</td>
<td>.1</td>
<td>.14</td>
</tr>
<tr>
<td>Parker Tract</td>
<td>33.05 76.91</td>
<td><em>Pinus taeda</em></td>
<td>.94 (.27)</td>
<td>1.1 (.42)</td>
<td>.91 (.45)</td>
<td>.08</td>
<td>.1</td>
<td>.09</td>
</tr>
<tr>
<td>Jones Center</td>
<td>35.81 76.67</td>
<td><em>Pinus palustris</em></td>
<td>1.4 (.24)</td>
<td>1 (.35)</td>
<td>5 (.27)</td>
<td>.09</td>
<td>.22</td>
<td>.21</td>
</tr>
<tr>
<td>Crosett</td>
<td>31.22 84.48</td>
<td><em>Pinus taeda</em></td>
<td>1.3 (.26)</td>
<td>1.7 (.49)</td>
<td>1.3 (.34)</td>
<td>.25</td>
<td>.31</td>
<td>.34</td>
</tr>
</tbody>
</table>

The slope parameter $m$ represents the change in $g_c$ as a function of $D^{1/2}$ (i.e. Eqn. 8). The number in parentheses shows the coefficient of determination (i.e. $R^2$) of that regression. Nocturnal $E_{sf}$ is that observed from 0 to 700 h and 1900 to 2300 h. NS, not significant; MI, $E_{sf}$ is monotonically increasing with vapor pressure deficit ($D$); PK, $E_{sf}$ peaks at some intermediate value of $D$; ST, $E_{sf}$ is stationary for much of the day; NC, no visible change in the hysteresis between $g_{c, sf}$ and $D$ at hourly timescales; I, hysteresis between $g_{c, sf}$ and $D$ at hourly timescale increases for drier conditions; D, hysteresis between $g_{c, sf}$ and $D$ at hourly timescale decreases for drier conditions; D, demand limitation; H, hydraulic limitation; non-S, non-stomatal limitation; STORAGE, a potential reliance on stored water. $\Delta m$ represents the change in $g_c$ as a function of $D^{1/2}$ (i.e. Eqn. 8). $\Delta m$ represents the change in $g_c$ as a function of $D^{1/2}$ (i.e. Eqn. 8). $\Delta m$ represents the change in $g_c$ as a function of $D^{1/2}$ (i.e. Eqn. 8).

*aWhich site species were associated with significantly different $m$ between dry and wet conditions and int and wet conditions ($p < 0.05$, Student's $t$-test).
and hydraulic architecture. Nonetheless, the qualitative nature of the relationships between gas exchange variables and $D$ should be preserved regardless of the numerical values of the parameters.

Despite these limitations, this study presents an approach for explicitly linking optimality constraints, hydraulic constraints and non-stomatal considerations in a unified model framework. The coherent mapping of the model predictions to the empirical observations described in this study is encouraging and can inform and motivate ongoing efforts to explore how these different mechanisms jointly or independently regulate leaf-level gas exchange during drought. Such efforts are necessary given the relatively large degree of uncertainty associated with model formulations for $g_c$ (Dietze et al. 2014) and the growing need for forest management activities that mitigate the deleterious effects of drought at species and ecosystem scales (Ford et al. 2011; Grant et al. 2013).

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**REFERENCES**


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**Figure 6.** The top row shows a comparison of measured stomatal conductance inferred from sap flow observations ($g_{c, SF}$) and modelled stomatal conductance ($g_c$) for two species growing the Duke Forest. The model was driven with species-specific parameterizations for the relationship between $K$ and $\Psi_L$, and species-specific parameterizations for $\mu_{WW}$. The sensitivity of $K$ to $\mu_{WW}$ (i.e. $\beta_K$) and $\Psi_{CRIT}$ determining the hydraulic limitation were varied as indicated in the legend. Data represent the daily averaged midday (1200–1500) stomatal conductance after normalizing by the value of $g_{c, sf}$ and $g_c$ during well-watered conditions. The bottom row shows the simulated daily averaged midday $\Psi_L$ as a function of daily averaged soil water potential ($\Psi_S$). The xylem air entry point ($\Psi_{AEP}$) and the leaf water potential associated with a 50% loss in $K$ ($\Psi_{S_{50}}$) are also shown for reference as dashed lines.


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