Draft manuscript for resubmission to *Agricultural and Forest Meteorology*

The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis

Remko A. Duursma[a], Craig V.M. Barton[a], Yan-Shih Lin[b], Belinda E. Medlyn[b], Derek Eamus[c,e], David T. Tissue[a], David S. Ellsworth[a], Ross E. McMurtrie[d]

[a] Hawkesbury Institute for the Environment, University of Western Sydney, Locked Bag 1797, Penrith 2751, NSW, Australia.

[b] Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

[c] School of the Environmental Sciences, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia.

[d] School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia.

[c] The National Centre for Groundwater Research and Training, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia.

Abstract

Leaf transpiration rate (*E*) frequently shows a peaked response to increasing vapour pressure deficit (*D*). The mechanisms for the decrease in *E* at high *D*, known as the 'apparent feed-forward response', are strongly debated but explanations to date have exclusively focused on hydraulic processes. However, stomata also respond to signals related to photosynthesis. We investigated whether the apparent feed-forward response of *E* to *D* in the field can be explained by the response of photosynthesis to temperature (*T*), which normally co-varies with *D* in field conditions. As photosynthesis decreases with increasing *T* past its optimum, it may drive a decrease in g_s that is additional to the response of g_s to increasing *D* alone. If this additional decrease is sufficiently steep and coupling between *A* and g_s occurs, it could cause an overall decrease in *E* with increasing *D*. We tested this mechanism using a gas exchange model applied to leaf-scale and whole-tree CO_2 and H_2O fluxes measured on *Eucalyptus saligna* growing in whole-tree chambers. A peaked response of *E* to *D* was observed at both leaf and whole-tree scales. We found that this peaked response was matched by a gas exchange model only when *T* effects on photosynthesis were incorporated. Furthermore, at elevated [CO_2], *E* peaked at higher *D*. We hypothesize thatcould be explained by an increase in the *T* optimum for A, as frequently observed, however we found no support for a higher *T* optimum for *A* in elevated [CO_2] in this study. We conclude that field-based studies of the relationship between *E* and *D* need to consider signals related to changing photosynthesis in addition to purely hydraulic mechanisms.

Key-words: Stomatal control, temperature response, plant water use, elevated CO_2

Introduction

The response of transpiration rate (E) to vapour pressure deficit (D) is well characterized (Monteith 1995), but the mechanisms underlying the response are not yet fully understood. At low D, E increases approximately linearly with increasing D. Subsequently, E saturates with increasing D due to decreasing stomatal conductance (g_s). Frequently, but not always, a third phase in the E-D response is observed, in which E decreases at high D (see reviews by Monteith, 1995; Franks et al., 1997). This third phase of the response of E to D is termed the 'apparent feed-forward' response (Farquhar, 1978; Monteith, 1995; Franks et al., 1997), and has caused much debate because it is difficult to explain from simple stomatal mechanics.

If the response of stomata to increasing D was the result of feedbacks of transpiration on leaf water status alone, we would expect that E would level off with increasing D, rather than decreasing after reaching some maximum value (Farquhar, 1978). A number of authors have proposed hydraulic mechanisms to explain the apparent feed-forward response. Farquhar (1978) argued that a reduction in E at high D can occur if some leaf water loss occurs through the cuticle, and stomata respond to this water loss. In support of this argument, Eamus et al. (2008) confirmed that manipulations of the leaf epidermis (to increase cuticular conductance) affected stomatal responses to D, and showed that feedback processes were sufficient to explain the three phase behaviour (*sensu* Monteith, 1995).

An alternative explanation for the peaked response of E to D is a decrease in plant hydraulic conductance with increasing D (Oren et al., 1999; Macfarlane et al., 2004) possibly as a result of cavitation of xylem due to high evaporative demand at high D, or drying soils. Dewar (2002) used a model of this mechanism to explain the reduction in E at high D, and Buckley (2005) provides additional model support for this hypothesis.

In this paper, we put forward a potential additional explanation for the apparent feed-forward response, which is based on the observation that stomata respond not only to leaf water status, but also to signals related to photosynthesis. The exact nature of these signals is not yet understood (Mott et al., 2009; Busch 2013), so they are represented minimally (if at all) in mechanistic models of stomatal conductance (Buckley and Mott 2013). It is well established experimentally that photosynthesis (A) and g_s both respond in parallel to changes in many environmental variables. In many cases, changes in photosynthetic (A) capacity can lead to concomitant changes in g_s (Wong et al. 1979, Messinger et al., 2006). This observation has been observed to hold for a wide range of stress responses including photoinhibition (Wong et al., 1985), ozone and acid mist (Barnes et al., 1990), chilling stress (Martin et al., 1981) high temperature stress (Hamerlynck and Knapp, 1996), salt stress (Seemann and Critchley, 1985) and transplanting stress (Guehl et al., 1989), but not for oxygen concentration (Farquhar and Wong 1984), nor does g_s decrease in plants where the Rubisco content has been experimentally reduced (see Busch 2013).

Since stomatal conductance responds to changes in photosynthetic capacity, hydraulic responses of E to D may be modulated by photosynthetic effects on g_s if photosynthetic capacity is changing at the same time. In field conditions, rising D is generally accompanied by a rise in air temperature (T), which directly affects photosynthetic capacity. Although there are some experiments that have demonstrated a peaked response of E to D when T is held constant (Eamus et al., 2008; Franks et al., 1997; Grantz, 1990; Thomas and Eamus 1999) most reports of the apparent feed-forward phenomenon are from studies where both D and T varied. These include field studies (Macfarlane et al., 2004; Meinzer et al., 1997; Pataki et al., 2000; Whitley et al., 2009), and early laboratory studies (West and Gaff, 1976). Thus, reports of a peaked E response are more common when T co-varies with D, than when T is

held constant. This accords with the view held by Franks (1997) that a peaked response of *E* to *D* is often difficult to demonstrate in laboratory conditions.

If *T* increases with *D*, there are consequences for photosynthesis and therefore for *E*. Experiments have shown that g_s responds strongly to *T* when *D* is held constant : it increases with *T* when *T* is below the photosynthetic optimum (Fredeen and Sage, 1999; Duursma et al., 2013), but decreases when *T* is above the photosynthetic optimum (Pons & Welschen, 2003). To explain the apparent feed-forward response, we can hypothesize that, as *D* and *T* increase, *A* declines past the photosynthetic temperature optimum, which leads to a decline in g_s that is additional to the direct effect of *D* on g_s and ultimately contributes to the decrease in *E*.

We tested this hypothesis against whole-tree flux and leaf-level gas exchange data from *Eucalyptus saligna* trees growing in whole-tree chambers. The data demonstrate a strong apparent feed-forward effect, with decreases in measured *E* at high *D*. As the weather conditions in the chambers tracked ambient, there was a strong correlation between *D* and air temperature.

We compared these data against leaf gas exchange models based on the well-known Ball-Berry-Leuning model of stomatal conductance (Leuning, 1995):

$$g_s = g_0 + g_1 \frac{A}{C_a} \cdot f(D) \qquad (1)$$

where C_a is the atmospheric CO_2 concentration (we assume that at the leaf surface $[CO_2]$ equals Ca, which is a good approximation in well-mixed conditions) g_1 is a constant parameter, and *f*(*D*) represents the effects of *D* on g_s. In this model, the effects of *T* on g_s operate through the dependence of *A* on *T* (Collatz et al., 1991). This dependence successfully combines the effects of *T* and *D* on g_s (Leuning, 1995). Depending on the form

chosen for *f*(D), some versions of this model (e.g. Leuning, 1995) predict a peaked response of *E* to *D* when *T* is constant, but other versions (e.g. Ball et al., 1987; Medlyn et al., 2011) do not.

To test our hypothesis that the photosynthetic response to *T* explains the apparent feed-forward response in these field data, we applied both the Leuning (1995) and Medlyn et al. (2011) versions of this model to the data, firstly assuming that temperature does not affect *A*, and then including the temperature dependence of *A*. By comparing the models without the temperature effect, we are able to determine whether the apparent feedforward effect described in the Leuning (1995) model is sufficient to explain the observed *D* response on its own. By then including the temperature effect on *A* in the models, we are able to determine to what extent the temperature effect on *A* is involved in the observed response to *D*. We do this for a unique model system where *A* and *E* are continuously measured for whole trees in outdoor enclosures.

Materials and methods

Whole-tree fluxes of CO_2 and H_2O

We use whole-tree flux measurements from the Hawkesbury Forest Experiment (HFE) (see Barton et al., 2010, for a detailed description). Twelve 10-m tall whole-tree chambers were established in 2006, and a single Sydney blue gum (*Eucalyptus saligna* Sm.) tree was planted in each chamber in April 2007. Final harvest occurred in March 2009. The experiment was a crossed C_a x drought design with three chambers in each of four treatments. Here, we use only the well-watered chambers. The C_a treatments were ambient (*ca*. 380 ppm; aC_a) and ambient + 240 ppm (eC_a). The chambers were climate-controlled; excellent control of

temperature and, to a slightly lesser extent, relative humidity, was achieved (Barton et al., 2010; 2012). Chambers were maintained with T_{air} equal to ambient air temperature outside chambers.

Whole-tree fluxes of CO_2 and H_2O were measured for each chamber at 14-minute intervals, along with measurements of air temperature (T_{air}) and vapour pressure deficit (D) inside the chambers. Full details of the measurements are provided in Barton et al. (2010). Photosynthetically active radiation (PAR) was measured outside the chambers. We use all available chamber flux data between 14 April 2008 and 5 March 2009, which consists of a near continuous record apart from a period of *ca*. seven weeks (August – September 2008) when chamber heights were extended (Barton et al., 2012). We averaged the 14-minute readings over hourly intervals. We also averaged the fluxes by C_a treatment for illustration of the patterns, but for analysis we used hourly averages by tree only. We used only the well-watered trees in the experiment (n=3 for both C_a treatments), and only data where the photosynthetically active radiation (PAR) was over 600 μmol m^{-2} s^{-1}, because we are here interested in behavior at high D, when PAR is near-saturating. All fluxes are expressed on a per unit leaf area basis, using estimates of total tree leaf area based on a combination of complete leaf counting (April 2008), destructive harvest (March 2009), and repeated measurements of height growth and litter fall (see Barton et al., 2012).

Leaf gas exchange

To confirm that responses at the leaf scale were similar to those observed for whole-tree fluxes, we analyzed T response curves of leaf gas exchange. These measurements were part of full A-C_i response curves, but here we only use the data when C_a was set to ambient conditions (*ca.* 380 ppm), which was always the first measurement. We used a LI-6400

portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA), with the LED light source set to 1800 µmol m^{-2} s^{-1}. Measurements were conducted at three or four leaf temperatures (15, 25, 32 and/or 36 °C, in that order) for all twelve chambers in November 2008 (i.e. before the drought treatment). There was no additional control of *D*, so that *D* and *T* co-varied in a similar way to Fig. 1. Erroneous data for one chamber were discarded.

Coupled leaf gas exchange model

We used a standard coupled leaf gas exchange model, using the photosynthesis model of Farquhar, von Caemmerer & Berry (1980), and a new stomatal model (Medlyn et al., 2011) which is very similar to a Ball-Berry type model, but also incorporates the idea that stomata are regulated to minimize the amount of transpiration per unit carbon gain. This model does not predict a feed-forward response of g_s to *D* if temperature is held constant. The model for g_s is given by:

$$g_s = g_0 + 1.6\left(1 + \frac{g_1}{D^{1-k}}\right)\frac{A}{C_a} \quad (2)$$

where g_0 is the residual conductance (g_s when *A* is zero), g_1 is a parameter related to the marginal water cost of carbon ($\lambda = \partial E/\partial A$), *k* an empirical parameter (that equals 0.5 when the response of g_s to *D* is optimal, see Duursma et al., 2013), C_a the atmospheric [CO_2] (ppm), and *D* the vapour pressure deficit (kPa). In the current study we found a robust way of estimating parameters of Eq. (2) using non-linear regression was to rearrange the equation with A/g_s as the dependent variable. Estimated parameter values obtained using the hourly whole-tree chamber flux data were g_0 = 0.014 mol m^{-2} s^{-1} (SE 0.0013), g_1 = 2.44 (SE 0.058) and *k* = 0.66 (SE 0.019).

For comparison, we also used the model of Leuning (1995), given by Eq. (3).

$$g_s = g_0 + g_1 \frac{A}{(C_a - \Gamma)(1 + D/D_0)} \quad (3)$$

We assume that the CO_2 compensation point (Γ) is zero, to be more comparable to Eq. (2). We used this model because, unlike Eq. (2), it does predict a peaked response of E to D. However, we found that when the model was fit to data, we did not observe a decrease in E with D when D < 5kPa (see Appendix B). For clarity, we only present the results using Eq. (2).

The widely used photosynthesis model of Farquhar et al. (1980) is not described here, see for example Medlyn et al. (2002). We use the temperature sensitivity of the maximum electron transport rate (J_{max}) and the maximum rate of Rubisco activity (V_{cmax}) as parameterized for *E. saligna* with a method equivalent to that of Lin et al. (2013), by measuring A-C_i response curves at various leaf temperatures. The parameters V_{cmax} and J_{max} at a standard leaf temperature of 25 °C were estimated from standard A-C_i curves (D. Ellsworth, unpublished data ; see Ellsworth et al., 2012, for a description of the methods used).

Dark respiration (R_d) was estimated using Eq. (4), which was parameterized based on Crous et al. (2011).

$$R_d = R_{d0} Q_{10}^{(T-25)/10} \quad (4)$$

Where R_{d0} is the basal respiration rate at T_{air}=25 °C. All parameter values are summarized in Table 1. We did not attempt to simulate the difference between T_{leaf} and T_{air}, because we lack estimates of boundary layer conductance inside the chambers. We assume throughout that T_{leaf} is equal to T_{air}, which does not affect the main results, but it does affect the location of the T_{air} optimum of E. If $T_{leaf} - T_{air} > 0$, the T_{air} optimum for E is lower by $T_{leaf} - T_{air}$, because T_{leaf} drives both photosynthetic capacity and D.

We simulated the whole-tree fluxes as if the tree behaves as a single leaf. The only adjustment we made was to reduce V_{cmax} and J_{max} (both set to 35% of their leaf-level estimates), to approximately fit the observed whole-tree flux data. We did not attempt to optimize the fit of the model to the data, as the objective was only to demonstrate the responses of A and E to D and T_{air}. We also used the MAESPA model (Duursma & Medlyn 2012) to simulate the whole-tree fluxes based on a more rigorous scaling of leaf-level gas exchange to canopy totals. The MAESPA results are not shown because they were qualitatively the same (and quantitatively similar) as simulations of the single-leaf model.

Data analysis

For the hourly whole-tree flux data, we used generalized additive models with automated smoothness selection (package *mgcv* in R 3.0.1; R Development Core Team, 2012) (Wood, 2006) to visualize the trends in A with T and E with D and the differences between C_a treatments, using the C_a-averaged flux data. This method does not assume a prior shape of the functional relationships between the variables.We also fit the generalized additive model by whole-tree chamber, from which the location of the peak was estimated. The locations of the peaks are referred to as T_{opt} (T where A is maximum) and D_{opt} (D where E is maximum). To test whether D_{opt} and T_{opt} differed with C_a treatment, we used a two-sample t-test assuming equal variance (with n=3). For the leaf gas exchange data, we fitted a second order polynomial to estimate T_{opt} for E and A, with a linear-mixed effects model (package *nlme* in R). From these fits, we used the delta method as implemented in the *car* package (Fox and Weisberg, 2010) to estimate an approximate 95% confidence interval for D_{opt} and T_{opt}.

Results

Using the coupled leaf gas exchange model parameterized for *E. saligna*, we modelled A and E along a range of temperatures (T), while at the same time increasing D using the empirical relationship shown in Fig. 1. As expected, A showed a peaked response to T (Fig. 2A, T_{opt} = 27.9 °C), and to D (Fig. 2B, D_{opt} = 1.7 kPa). Because the g_s model we used (Eq. (1)) assumes a strong coupling between g_s and A, E also showed a peaked response to T and D (Fig 2A and 2B). The T_{opt} for E was much higher than for A (34.2 °C). Similarly, the D_{opt} was higher for E than A (2.8 kPa). Simulations of g_s demonstrated that very different results were obtained when only D was varied, or when D and T co-varied (Fig. 2C). In the latter case, g_s showed a much more rapid decline at high D and demonstrated the characteristic three-phase response.

The whole-tree CO_2 flux expressed on a per unit leaf area basis (A_{tree}) showed a peaked response to air temperature (T_{air}) (Fig. 3), and D (Fig. A1). A_{tree} declined to near zero when T_{air} was *ca*. 45 °C. The leaf gas exchange model used either the measured co-variation in D and T_{air} (based on Fig. 1), or used only T_{air} as a driver (with D constant at 1.5 kPa). Results of the two simulations were similar (Fig. 3C), demonstrating that the T_{air} response of A_{tree} was primarily due to direct T_{air} effects (which affects V_{cmax} , J_{max}, their kinetics, and R_d); the influence of increasing D when applied with increasing T was barely evident (Fig 3c). The coupled leaf gas exchange model showed a peaked response in A, and an increase in T_{opt} with elevated C_a (from 27.1 to 30.0 °C) (Fig. 3C). The flux data did not show a significant increase in T_{opt}, as concluded from the tree-level fluxes (Fig. 5A, p = 0.129).

The whole-tree fluxes of H_2O, expressed per unit leaf area (E_{tree}), also showed a peaked response to D (Figs. 4A and 4B) and T_{air} (Fig. A2). The leaf gas exchange model was used to predict E_{tree} as a function of either D alone (with T_{air} at 25 °C) or with D and T_{air} co-varying (Fig 4C; based on the empirical relationship in Fig. 1). The simulated responses of E_{tree} to D alone differed between the two simulations: the peaked response in E_{tree} only appeared when T_{air} was taken into account, because it drives A_{tree} when T_{air} exceeds the photosynthetic

optimum (Fig. 4C). In the gas exchange model, D_{opt} for *E*increased with elevated C_a (from 2.4 to 2.9 kPa). This result was also observed when we used a different stomatal conductance model, that of Leuning (1995) (Eq. (2)) (Fig. B1). The flux data also showed an increase in Dopt with eC_a (Fig. 5B, p=0.028), from 2.2 to 2.8kPa, similar to the gas exchange model.

To test whether the response of whole-tree fluxes to *D* and T_{air} were similar to those at the leaf level, we used the leaf gas exchange data to determine T_{leaf} responses of *A* and *E*, while *D* was co-varying naturally (Fig. 6). The response of *E* to *D* was qualitatively similar to the whole-tree flux data and simulations, with *E* reaching a maximum value at a *D* of 2.45 kPa for ambient C_a (95% CI : 2.22 – 2.69) or 2.91 kPa for elevated C_a (95% CI : 2.44 – 3.38). Although this shift in optimum *D* is consistent with our expectation, the difference was not significant (P > 0.1) as the curve was broader at the leaf-level than for the canopy.

Discussion

Using whole-tree flux and leaf-level gas exchange data on *Eucalyptus saligna*, we demonstrated a strong decrease in *E* at high *D*. We advanced a novel hypothesis for the explanation of the peaked response of *E* to *D*, based on the strong correlation between T_{air} and *D* in field conditions, and the assumption that g_s is linked to photosynthetic rate. We argue that the coupling between g_s and photosynthetic rate was necessary to fully explain the response of *E* to *D* in field conditions. This assumption is reasonable based on apparent coupling of *A* and g_s that is employed in Ball-Berry type stomatal models (Leuning, 1995) and has broad experimental support (Wong and Farquhar, 1979), although the nature of this coupling is still under debate (Busch 2013). When T_{air} increases above the optimum for photosynthesis, the decrease in photosynthesis causes a decrease in g_s. If this decrease in g_s with increasing *D* is steep enough, *E* declines. A coupled leaf gas exchange model

incorporating the photosynthetic temperature dependence was successful in predicting the response of *E* to *D* observed in a whole-tree chamber experiment.

We stress that our hypothesis to explain the peaked response of *E* to *D* requires that *D* and *T* are correlated, as is always the case in field conditions, but not always in laboratory experiments. For example, a number of studies have demonstrated this 'apparent feed-forward' behaviour when *D* varied but *T* was held constant (Grantz, 1990; Bunce, 1997; Eamus et al., 2008). Our hypothesized mechanism likely explains many field observations of the peaked response of *E* to *D*, as *D* and *T* will be nearly always correlated, and photosynthesis responds strongly to temperature (Berry and Björkman, 1980; Medlyn et al., 2002). A field study in a native *Eucalyptus* woodland (Whitley et al., 2008) showed a peaked response of canopy-scale transpiration (estimated from sap flow) to *D*, with the optimum in the range 2-3 kPa, consistent with our results (Figs. 2 & 4). Some laboratory experiments also allowed *D* and *T* to covary. For example, a study used by the review in Farquhar (1978) to demonstrate feed-forward mechanisms (West and Gaff, 1976) allowed *D* to co-vary with *T*. We suggest that in those studies, the effect of *T* on photosynthesis may explain the peaked response of *E* to *D*.

Our hypothesized mechanism for the peaked *E* response does not preclude other mechanisms from operating at the same time. For example, it is possible that hydraulic conductance decreases at high *D*, which can cause a decrease in *E* at high *D* (see Buckley, 2005). In our experiment, we lacked the data to test this specific hypothesis. Other hypothesized mechanisms include feedbacks associated with epidermal water relations (Eamus et al., 2008), and a novel mechanism arising from a model that assumes the guard cell equilibrates with the water vapour inside the leaf (Peak & Mott, 2011; Mott & Peak, 2013). These, and

perhaps other mechanisms, may operate alongside a photosynthetically-driven decline in *E*. It has yet to be demonstrated which mechanisms, including the one we propose, are most important in describing the decline in *E* at high *D*.

Although we argue that the photosynthetic *T* optimum causes an optimum in the response of *E* to *D*, this should not be taken to mean the optimum occurs at the same *T*. In fact, *E* peaks at a higher *T* than *A* or g_s (Fig. 1, see also Ku et al., 1977). This can be explained by assuming that, in a well-stirred cuvette, $E = g_s D$. When g_s is exactly proportional to $1/D$, then it is easy to see that *E* remains constant as *D* is increasing. Therefore, for *E* to decrease with increasing *D*, g_s needs to decrease with a slope that is steeper than $1/D$. As a result, the *D* at which maximum *E* occurs has to occur at a higher *D* than that for maximum *A*.

The coupled leaf gas exchange model demonstrated that an increase in the optimum *T* for photosynthesis in elevated C_a can result in an increase in the *D* optimum for *E* (Figs. 3C & 4C), and this increase was confirmed for the whole-tree flux data (Fig. 5B). However, the observed increase in the *T* optimum for *A* was not statistically significant for either whole-tree flux data, or leaf gas exchange data ($P > 0.05$ for both), because there was substantial scatter around the location of the optimum. An increase in T_{opt} with eC_a has been observed in leaf-scale measurements of *A* (e.g. Eamus et al., 1995), including for our study species *E. saligna* (Ghannoum et al., 2010). With increasing *T*, oxygenation by Rubisco is increasingly favoured over carboxylation, so that the amount of carbon lost through photorespiration increases with *T*. Because elevated C_a decreases oxygenation by increasing $[CO_2]$ within the chloroplast, this effect diminishes under elevated C_a. As a result, the C_a stimulation of photosynthesis is larger at higher *T*. This mechanism is incorporated in the model of Farquhar et al. (1980) (see also Long, 1991; McMurtrie and Wang, 1993). However, it has not been previously suggested that this shift in T_{opt} with eC_a could also contribute to a shift in the *T* optimum for *E*. Targeted experiments where *D*, *T* and $[CO_2]$ are carefully controlled, and

varied in tandem or alone, will help clarify these relationships to the peaked response of E to D.

It is well known that elevated C_a can lead to a decrease in g_s and E (Medlyn et al., 2001). Our whole-tree flux data also demonstrated a decrease in E_{tree}, but only when D was less than *ca.* 2.5 kPa (Fig. 4) (see also Barton et al., 2012). When D was larger, eC_a did not decrease E_{tree}, and even led to an increase in some cases. This observation was matched by the model, when both T_{air} and D were varied (Fig. 4C). This pattern may be explained by the larger stimulation of photosynthesis at high T, which tends to counteract the stomatal closure arising from the effect of high D. These results show that predictions of the effects of elevated C_a on vegetation water use are highly dependent on the interactions with changes in T_{air}.

Conclusions

A better understanding of the mechanisms of the response of plant water use to atmospheric humidity and temperature would lead to improved model-based projections of climate change effects on vegetation water use and carbon uptake. Here we advance an hypothesis that explains the peaked response of E to increasing D, in a way that could readily be incorporated in models. Evidence for the role of temperature in controlling the response of E to D comes from an experiment on trees growing in elevated C_a, which increased the D optimum for E, consistent with the expectation that elevated [CO_2] increases the T optimum for A although we were unable to demonstrate this increase empirically.

It is difficult to link the D optimum of E to the T optimum of A across studies, because it requires also that we know how D was related to T, which is seldom reported.

Our explanation of the peaked *E* response provides additional evidence for the link between photosynthetic capacity and stomatal conductance, and helps to expand on the exclusively hydraulic framework so often used to explain stomatal responses to variation in *D*.

Acknowledgments

This research was supported by the NSW government Climate Action Grant (NSW T07/CAG/016), and funding by the Australian Government's Department of Climate Change for the Hawkesbury Forest Experiment. We thank Burhan Amiji and Michael Forster for outstanding technical support. The Hawkesbury Forest Experiment is made possible by a close collaboration with Professor Sune Linder (Swedish University of Agricultural Sciences, Sweden), who generously provided the whole-tree chambers.

Tables

Table 1. Parameter values used in the coupled leaf gas exchange model. For all simulations, photosynthetically active radiation (PAR) was set to 1500 μmol m^{-2} s^{-1}. Air (leaf) temperature was either varied or set to 25 °C, and vapour pressure deficit either varied or was set to 1.5 kPa. The values for parameters V_{cmax} and J_{max} are at 25 °C.

Parameter	Value	Units	Source
$\mathbf{V_{cmax}}$	89.5	μmol m^{-2} s^{-1}	D. Ellsworth (unpublished data)
$\mathbf{J_{max}}$	145.4	μmol m^{-2} s^{-1}	"
$\mathbf{g_0}$	0.014	mol m^{-2} s^{-1}	This study
$\mathbf{g_1}$	2.44		"
k	0.66		"
$\mathbf{R_{d0}}$	0.92	μmol m^{-2} s^{-1}	This study, based on Crous et al.,, 2011
$\mathbf{Q_{10}}$	1.95		"

373 **Figure captions**

374 **Figure 1.** The dependence of vapour pressure deficit (D) on air temperature (T_{air}) for the
375 chamber flux dataset (data are hourly averages, daylight period only). The thick solid line is
376 the fitted power function ($D = 0.000605 * T_{air}^{2.39}$). The dashed lines are estimates of D when
377 relative humidity (RH) is constant.

378 **Figure 2. A, B**. Simulated leaf-level transpiration (E) and CO_2 assimilation rate (A) using the
379 coupled leaf gas exchange model. For the simulations, D was allowed to co-vary with T_{leaf}
380 using the empirical relationship shown in Fig. 1. Note that the optimum T_{leaf} for E is higher
381 than the optimum T_{leaf} for photosynthesis. **C.** Simulated stomatal conductance (g_s) as a
382 function of D, either by varying both D and T_{leaf} (solid line, same simulations as in panels A
383 and B), or only D (dashed line, with T_{leaf} set to 25 °C).

384 **Figure 3. A.** Measured hourly whole-tree CO_2 flux rates (A_{tree}) as a function of chamber air
385 temperature (T_{air}), for ambient and elevated C_a treatments. **B.** Smoothed regression (see
386 Methods) of the data, showing estimates of the T_{air} at which A_{tree} is optimum as vertical lines.
387 **C.** Simulations of A_{tree} using the coupled leaf gas exchange model (and reduced V_{cmax} and
388 J_{max}, see Methods). The simulations varied both T_{air} and D (solid line) or D only (dashed
389 line).

390 **Figure 4. A.** Measured hourly whole-tree H_2O flux rates (E_{tree}) as a function of chamber air
391 vapour pressure deficit (D), for ambient and elevated C_a treatments. **B.** Smoothed regression
392 (see Methods) of the data. **C.** Simulation of E_{tree} using the coupled leaf gas exchange model
393 (and a reduced V_{cmax} and J_{max}, see Methods). The simulation varied both T_{air} and D (solid
394 line) or D only (dashed line).

395

Figure 5. Relationships between E_{tree} and D (panel A), and A_{tree} and T_{air}, shown as smoothed regressions (from a generalized additive model fit) fitted by whole-tree chamber. The filled circles indicate the optimum E_{tree} or A_{tree}, the grey areas are approximate 95% confidence intervals for the mean.

Figure 6. Leaf-level measurements of CO_2 assimilation (A) and transpiration (E) as a function of T_{air} or D. Individual points are means for a chamber at a particular T_{air} setting of 15, 25, 32 and/or 36 °C (usually three T_{air} settings per chamber). Solid lines are second order polynomial fits (the quadratic term was always significant, $P < 0.05$).

Appendix A

Figure A1. Measured hourly whole-tree CO_2 flux rates (A_{tree}) as a function of vapour pressure deficit (D) inside the chamber, for ambient and elevated C_a treatments. **B.** Smoothed regression (see Methods) of the data **C.** Simulation of A_{tree} using the coupled leaf gas exchange model (and reduced V_{cmax} and J_{max}, see Methods). The simulation varied both T_{air} and D (solid line) or D only (dashed line).

Figure A2. Measured hourly whole-tree H_2O flux rates (E_{tree}) as a function of chamber air temperature (T_{air}), for ambient and elevated C_a treatments. **B.** Smoothed regression (see Methods) of the data. **C.** Simulation of E_{tree} using the coupled leaf gas exchange model (and reduced V_{cmax} and J_{max}, see Methods). The simulation varied both T_{air} and D (solid line) or D only (dashed line).

Appendix B

Figure B1. Comparison of two stomatal conductance models and their predictions of the E vs. D dependence (BBOpti, Medlyn et al. 2011; BBLeuning, Leuning 1995). Although the

Leuning (1995) model can predict a decrease in E at high D (Dewar 1995), we never observed a peak in the range of observed D (0 – 5.5 kPa) unless g_0 was set to zero, and D_0 to an arbitrarily low value. In the latter case, the model fit very poorly at low D. Parameters values were, g_1 = 6.63, g_0 = 0.014, D_0 = 5.01.

References

Ball J.T., Woodrow I.E., Berry J.A,. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: Progress in photosynthesis research (ed J. Biggins), pp. 221-224. Martinus-Nijhoff Publishers, Dordrecht, the Netherlands.

Barnes J., Eamus D., Brown K., 1990. The influence of ozone, acid mist and soil nutrient status on Norway spruce [Picea abies (L.) Karst.]. New Phytol. 114, 713-720.

Barton C.V.M., Duursma R.A., Medlyn B.E., Ellsworth D.S., Eamus D., Tissue D.T., Adams M.A., Conroy J., Crous K.Y., Liberloo M., Löw M., Linder S., McMurtrie R.E. ,2012. Effects of elevated atmospheric [CO_2] on instantaneous transpiration efficiency at leaf and canopy scales in Eucalyptus saligna. Global Change Biol. 18, 585-595.

Barton C.V.M., Ellsworth D.S., Medlyn B.E., Duursma R.A., Tissue D.T., Adams M.A., Eamus D., Conroy J.P., McMurtrie R.E., Parsby J., Linder S., 2010. Whole-tree chambers for elevated atmospheric CO_2 experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. Agric. For. Meteorol. 150, 941-951.

Berry J., Björkman O., 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Rev. Plant Physiol. 31, 491-543.

Buckley T.N., 2005. The control of stomata by water balance. New Phytol. 168, 275-291.

Buckley, T.N., Mott, K.A., 2013. Modelling stomatal conductance in response to environmental factors. Plant Cell Environ. 36, 1691–1699.

Bunce J.A., 1997. Does transpiration control stomatal responses to water vapour pressure deficit? Plant Cell Environ. 20, 131-135.

Busch, F.A., 2013. Opinion: The red-light response of stomatal movement is sensed by the redox state of the photosynthetic electron transport chain. Photosynthesis Res. DOI 10.1007/s11120-013-9805-6.

Collatz G., Ball J., Grivet C., Berry J., 1991. Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. Agric. For. Meteorol. 54, 107-136.

Crous K.Y., Zaragoza-Castells J., Löw M., Ellsworth D.S., Tissue D.T., Tjoelker M.G., Barton C.V.M., Gimeno T.E., Atkin O.K. ,2011. Seasonal acclimation of leaf respiration in Eucalyptus saligna trees: impacts of elevated atmospheric CO_2 and summer drought. Global Change Biol. 17, 1560-1576.

Dewar, R., 1995. Interpretation of an empirical model for stomatal conductance in terms of guard cell function. Plant Cell Environ. 18(4), 365-372.

Dewar R. ,2002. The Ball-Berry-Leuning and Tardieu-Davies stomatal models: synthesis and extension within a spatially aggregated picture of guard cell function. Plant Cell Environ.. 25, 1383-1398.

Doughty C.E., Goulden M.L. ,2008. Are tropical forests near a high temperature threshold. J Geophys. Res. 113, G00B07.

Duursma R.A., Medlyn B.E., 2012. MAESPA: a model to study interactions between water limitation, environmental drivers and vegetation function at tree and stand levels, with an example application to [CO_2] × drought interactions. Geosci. Model Dev. 5, 919-940.

Duursma R.A., Payton P., Bange M.P., Broughton K.J., Smith R.A., Medlyn B.E., Tissue D.T., 2013. Near-optimal response of instantaneous transpiration efficiency to vapour pressure deficit, temperature and [CO_2] in cotton (*Gossypium hirsutum* L.). Agric. For. Meteorol. 168, 168-176.

Eamus D., Duff G.A., Berryman C.A., 1995. Photosynthetic responses to temperature, light flux-density, CO_2 concentration and vapour pressure deficit in *Eucalyptus tetrodonta* grown under CO_2 enrichment. Environ. Pollution. 90, 41-49.

Eamus D., Taylor D.T., Macinnis-Ng C.M.O., Shanahan S., De Silva L., 2008. Comparing model predictions and experimental data for the response of stomatal conductance and guard cell turgor to manipulations of cuticular conductance, leaf-to-air vapour pressure difference and temperature: feedback mechanisms are able to account for all observations. Plant Cell Environ. 31, 269-277.

Ellsworth D.S., Thomas R., Crous K.Y., Palmroth S., Ward E., Maier C., DeLucia E., Oren R., 2012. Elevated CO_2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. Global Change Biol. 18, 223-242.

Farquhar G., 1978. Feedforward responses of stomata to humidity. Fun. Plant Biol. 5, 787-800.

Farquhar G.D., Caemmerer S., Berry J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta, 149, 78-90.

Fox, J., Weisberg, S., 2010. An R companion to applied regression, 2nd ed. Sage Publications, Inc. 472p.

Franks P.J., Cowan I.R., Farquhar G.D., 1997. The apparent feedforward response of stomata to air vapour pressure deficit: information revealed by different experimental procedures with two rainforest trees. Plant Cell Environ., 20, 142-145.

Fredeen, A.L., Sage, R.F., 1999. Temperature and humidity effects on branchlet gas-exchange in white spruce: an explanation for the increase in transpiration with branchlet temperature. Trees 14, 161–168.

Ghannoum, O., N.G. Phillips, M.A. Sears, B.A. Logan, J.D. Lewis, J.P. Conroy, D.T. Tissue, 2010. Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO_2] and temperature. Plant, Cell Environ. 33(10): 1671-1681.

Grantz D.A., 1990. Plant response to atmospheric humidity. Plant Cell Environ., 13, 667-679.

Guehl J., Aussenac G., Kaushal P., 1989. The effects of transplanting stress on photosynthesis, stomatal conductance and leaf water potential in *Cedrus atlantica* Manetti seedlings: role of root regeneration. Ann. Sci. For. 46S, 464-468.

Hamerlynck E., Knapp A.K., 1996. Photosynthetic and stomatal responses to high temperature and light in two oaks at the western limit of their range. Tree Physiol. 16, 557-565.

Katul G.G., Palmroth S., Oren R., 2009. Leaf stomatal responses to vapour pressure deficit under current and CO_2-enriched atmosphere explained by the economics of gas exchange. Plant Cell Environ. 32, 968-979.

Ku S.B., Edwards G.E., Tanner C.B., 1977. Effects of light, carbon dioxide, and temperature on photosynthesis, oxygen inhibition of photosynthesis, and transpiration in *Solanum tuberosum*. Plant Physiol. 59, 868-872.

Leuning R., 1995. A critical-appraisal of a combined stomatal-photosynthesis model for C-3 plants. Plant Cell Environ. 18, 339-355.

Lin, Y.-S., Medlyn, B.E., De Kauwe, M.G. and Ellsworth, D.S., 2013. Biochemical photosynthetic responses to temperature: how do interspecific differences compare with seasonal shifts? Tree Physiology. doi: 10.1093/treephys/tpt047

Long S., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant Cell Environ. 14, 729-739.

Lösch R., 1977. Responses of stomata to environmental factors—experiments with isolated epidermal strips of *Polypodium vulgare*. I. Temperature and humidity. Oecologia. 29, 85-97.

Macfarlane C., White D.A., Adams M.A., 2004. The apparent feed-forward response to vapour pressure deficit of stomata in droughted, field-grown *Eucalyptus globulus* Labill. Plant Cell Environ. 27, 1268-1280.

Martin B., Ort D.R., Boyer J.S., 1981. Impairment of photosynthesis by chilling-temperatures in tomato. Plant Physiol. 68, 329-334.

McMurtrie R., Wang Y., 1993. Mathematical models of the photosynthetic response of tree stands to rising CO_2 concentrations and temperatures. Plant Cell Environ. 16, 1-13.

Medlyn B.E., Dreyer E., Ellsworth D., Forstreuter M., Harley P.C., Kirschbaum M.U.F., Le Roux X., Montpied P., Strassemeyer J., Walcroft A., Wang K., Loustau D., 2002. Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant Cell Environ. 25, 1167-1179.

Medlyn B.E., Duursma R.A., Eamus D., Ellsworth D.S., Prentice I.C., Barton C.V.M., Crous K.Y., De Angelis P., Freeman M., Wingate L., 2011. Reconciling the optimal and empirical approaches to modelling stomatal conductance. Global Change Biol. 17, 2134-2144.

Meinzer F.C., Hinckley T.M., Ceulemans R., 1997. Apparent responses of stomata to transpiration and humidity in a hybrid poplar canopy. Plant Cell Environ. 20, 1301-1308.

Messinger, S.M., Buckley, T.N., Mott, K.A., 2006. Evidence for Involvement of Photosynthetic Processes in the Stomatal Response to CO2. Plant Physiol. 140, 771–778.

Monteith J.L., 1995. A reinterpretation of stomatal responses to humidity. Plant Cell Environ. 18, 357-364.

Mott, K.A., 2009. Opinion: Stomatal responses to light and CO_2 depend on the mesophyll. Plant, Cell Environ., 32(11): 1479-1486.

Mott K.A., Peak D., 2013. Testing a vapour-phase model of stomatal responses to humidity. Plant Cell Environ. 36(5), 936-944.

Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N., Schäfer, K.V.R., 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant Cell Environ. 22, 1515–1526.

Pataki D.E., Oren R., Smith W.K., 2000. Sap flux of co-occurring species in a western subalpine forest during seasonal soil drought. Ecology. 81, 2557-2566.

Peak, D., Mott, K.A., 2011. A new, vapour-phase mechanism for stomatal responses to humidity and temperature. Plant Cell Environ. 34, 162–178.

Pieruschka R., Huber G., Berry J.A., 2010. Control of transpiration by radiation. PNAS. 107, 13372-13377.

Pons, T.L., Welschen, R.A.M., 2003. Midday depression of net photosynthesis in the tropical rainforest tree Eperua grandiflora: contributions of stomatal and internal conductances, respiration and Rubisco functioning. Tree Physiol. 23, 937–947.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rodriguez J.L., Davies W.J., 1982. The effects of temperature and ABA on stomata of *Zea mays* L. J Exp. Bot. 33, 977-987.

Sage R.F., Sharkey T.D., 1987. The effect of temperature on the occurrence of O_2 and CO_2 insensitive photosynthesis in field grown plants. Plant Physiol. 84, 658-664.

Seemann J.R., Critchley C., 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta. 164, 151-162.

Thomas D., Eamus D., 1999. The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant C_i and on stem hydraulic conductance and foliar ABA concentrations. J Exp. Bot. 50, 243-251.

West D., Gaff D., 1976. The effect of leaf water potential, leaf temperature and light intensity on leaf diffusion resistance and the transpiration of leaves of *Malus sylvestris*. Physiol. Plant. 38, 98-104.

Whitley R., Medlyn B., Zeppel M., Macinnis-Ng C., Eamus D., 2009. Comparing the Penman–Monteith equation and a modified Jarvis–Stewart model with an artificial neural network to estimate stand-scale transpiration and canopy conductance. J. Hydrol. 373, 256-266.

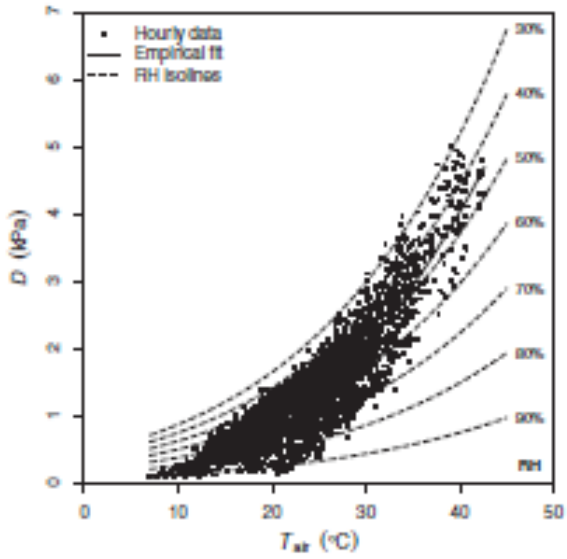
Whitley R., Zeppel M., Armstrong N., Macinnis-Ng C., Yunusa I., Eamus D., 2008. A modified Jarvis-Stewart model for predicting stand-scale transpiration of an Australian native forest. Plant and Soil. 305, 35-47.

Winter K., Aranda J., Garcia M., Virgio A., Paton S.R., 2001. Effect of elevated CO_2 and soil fertilization on whole-plant growth and water use in seedlings of a tropical pioneer tree, *Ficus insipida*. Flora. 196, 458-464.

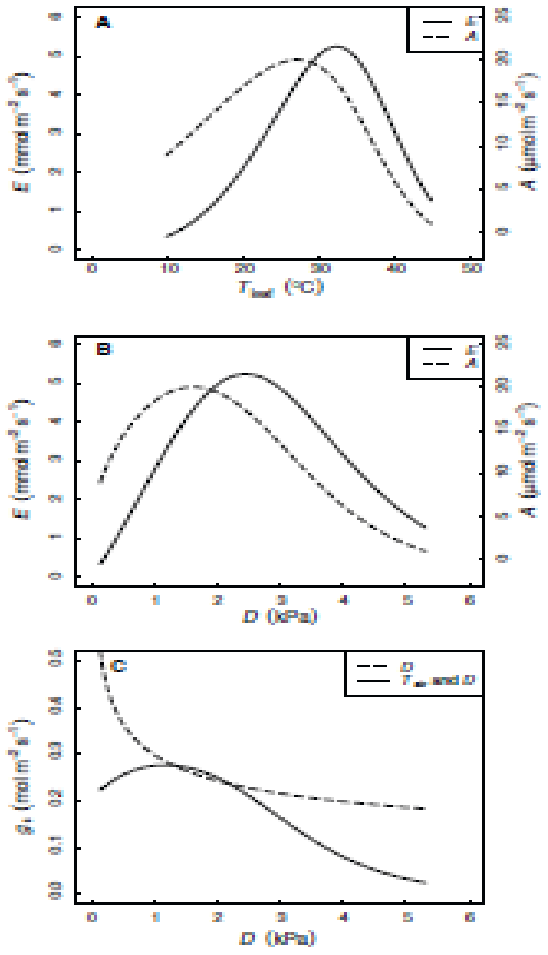
Wong S., Cowan I., Farquhar G., 1979. Stomatal conductance correlates with photosynthetic capacity. Nature, 282, 424-426.

Wong S.C., Cowan I.R., Farquhar G.D., 1985. Leaf conductance in relation to rate of CO_2 assimilation: III. Influences of water stress and photoinhibition. Plant Physiol. 78, 830.

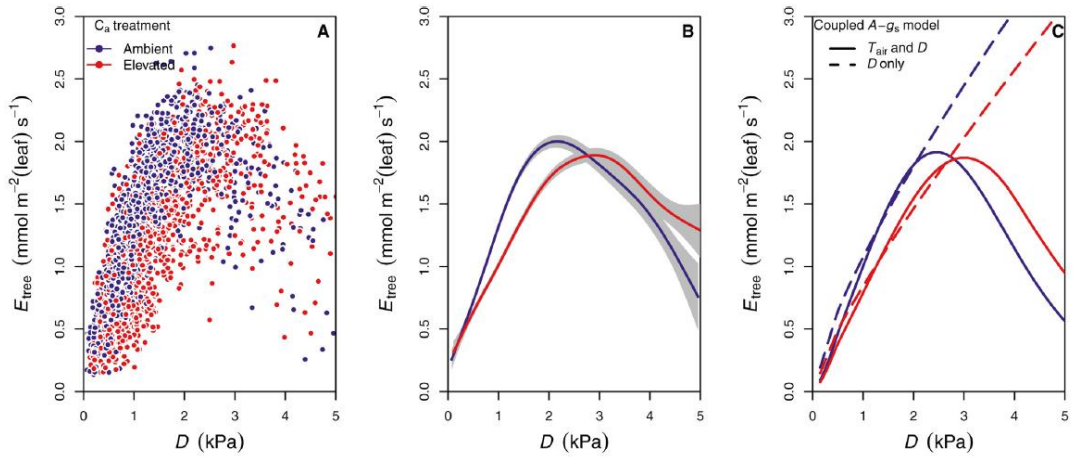
Wood S.N., 2006. Generalized additive models : an introduction with R. Chapman & Hall/CRC.

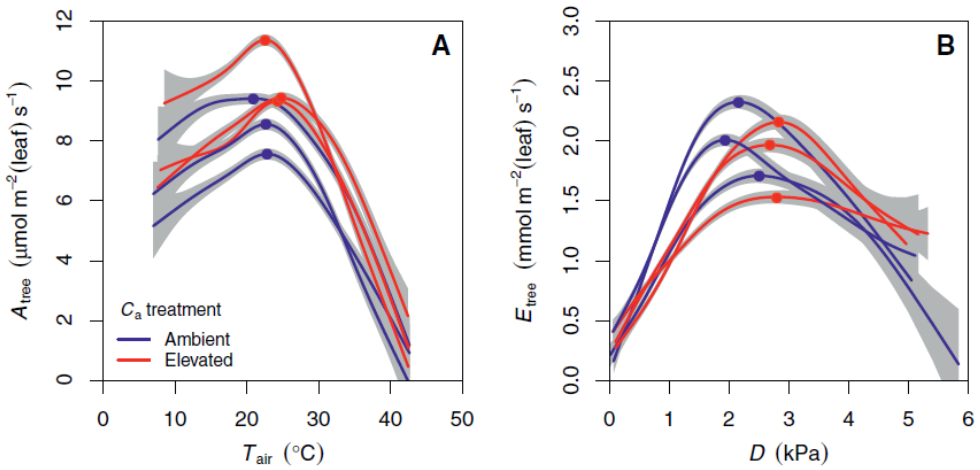


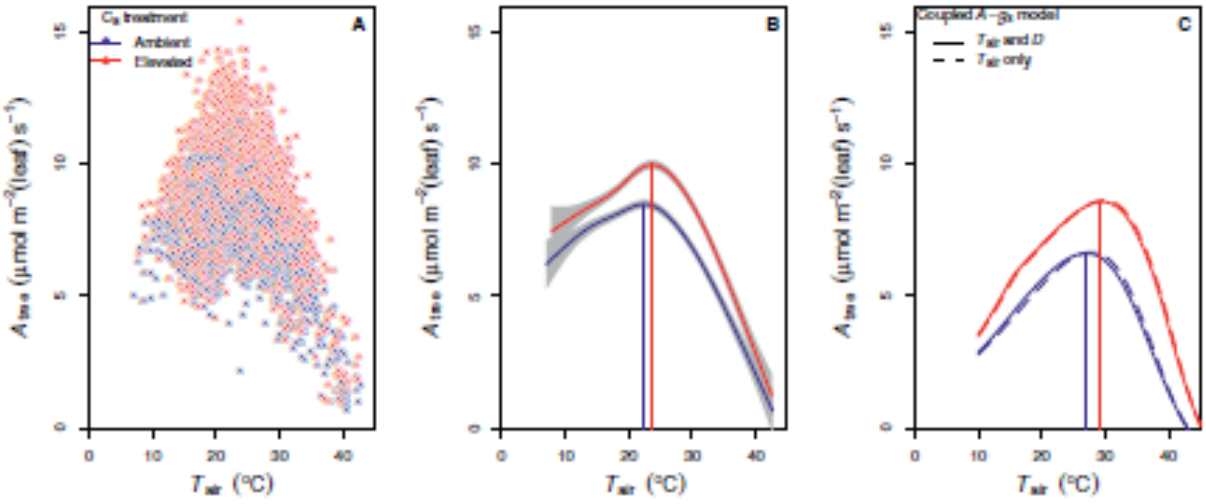
609
610
611
612
613
614
615

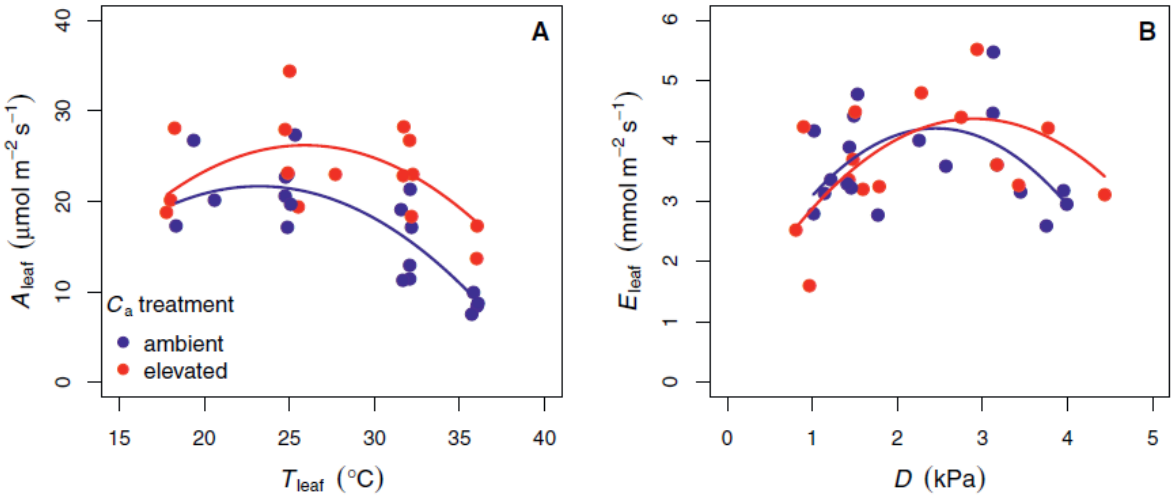


616
617
618


A
B
C
C_a treatment
Ambient
Elevated
Coupled A–g_s model
T_{air} and D
D only
E_{tree} (mmol m^{-2}(leaf) s^{-1})
D (kPa)


A
B
A_{tree} (µmol m^{-2}(leaf) s^{-1})
C_a treatment
Ambient
Elevated
T_{air} (°C)
E_{tree} (mmol m^{-2}(leaf) s^{-1})
D (kPa)


A
B
C
Ambient
Elevated
T_{air} (°C)


A
B
A_{leaf} (µmol m^{-2} s^{-1})
E_{leaf} (mmol m^{-2} s^{-1})
T_{leaf} (°C)
D (kPa)
C_a treatment
ambient
elevated

632