

# **Elevated CO<sub>2</sub> Effects On Vegetation: Informing Modelling Through Meta-Analysis And Targeted Experiments**

By

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## **STATEMENT OF CANDIDATE**

I certify that the work presented in this thesis entitled “Elevated CO<sub>2</sub> Effects On Vegetation: Informing Modelling Through Meta-Analysis And Targeted Experiments” has not been previously submitted for a degree, nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that this thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis did not require approval from Macquarie University Ethics Committee.

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## ABSTRACT

Atmospheric CO<sub>2</sub> concentration ( $C_a$ ) is currently increasing at an unprecedented rate and this increase has important effects on vegetation. During the last three decades, many experiments examined the response of vegetation to the rising  $C_a$ . However, the results from these experiments have not been fully incorporated into the global models used by the IPCC in predicting the future course of  $C_a$  and vegetation dynamics. This is partly due to the fact that experimental data are not often analysed in ways that directly relate to model formulations. Therefore, the main aim of this study was to link experimental data more directly with model formulations. The research involved (a) meta-analysis of experimental data using models as a framework and (b) experimental work explicitly designed to address model predictions.

In my thesis, I addressed several aspects of modeling  $C_a$  responses of vegetation. The first question I addressed was whether there is a temperature effect on plant response to elevated CO<sub>2</sub> ( $eC_a$ ). Because of the kinetics of the photosynthetic enzyme Rubisco, theory predicts that the  $C_a$  response should be greater at higher temperatures. Vegetation models incorporating these physiological responses predict that responses of photosynthesis, and consequently net primary productivity (NPP), to  $eC_a$  should increase with rising temperature, and be larger in warm tropical forests than in cold boreal forests. However experimental data do not always show such an interaction. I used meta-analysis techniques to test whether such an interaction is observed experimentally. Firstly, I tested for an interaction effect on plant growth responses in factorial  $eC_a$  x temperature experiments. This analysis showed a mean interaction effect size of 8.2% (95% CI -0.85% to 18.0 %.) for plant above-ground biomass. Although the interaction was not significantly different from zero, it was also not significantly different from the predicted interaction values obtained from leaf-level and canopy-level models. In the second meta-analysis, I examined  $eC_a$  experiments on woody plants across the globe to test for a relationship between the  $eC_a$  effect and mean annual temperature (MAT). This meta-regression analysis gave a positive slope that was again not significantly different from zero or from the slope predicted by global-scale models. With limited factorial studies and insufficient experimental data in tropical regions, there was a lack of statistical power to determine whether or not a positive interaction exists between  $eC_a$  and temperature.

The second question I addressed was how stomatal conductance of C4 plants behaves in response to changing  $C_a$ . Optimal stomatal theory says that stomata should act to maximize

carbon gain (photosynthesis,  $A$ ) while minimizing water loss (transpiration,  $E$ ). That is, the optimal stomatal behavior would be to maximise the integrated sum of  $(A - \lambda E)$ , where  $\lambda$  ( $\text{mol C mol}^{-1} \text{H}_2\text{O}$ ) represents the marginal carbon cost of water use. The unified stomatal conductance model by Medlyn et al. (2011) captures stomatal responses for the C3 plants. Since C4 plants have different photosynthetic  $C_a$  responses, we can expect different stomatal responses from them. By using optimal stomatal theory, I predicted how stomatal conductance of C4 plants should change with  $eC_a$ , and tested experimentally whether C4 plants showed this behavior. The theory predicted that stomata of C4 plants should be more sensitive to increasing  $C_a$  than C3 plants, however my experimental results showed that C4 plants followed the same stomatal behavior predicted for C3 plants.

Optimal stomatal theory also predicts that leaf-level water use efficiency (WUE) of plants should be proportional to  $C_a$ . However, whole-plant WUE is predicted to be somewhat less responsive than leaf-level WUE due to boundary layer effects on canopy transpiration. In the third chapter of my thesis I tested this prediction using meta-analysis techniques, statistically combining all previously published studies on increased  $C_a$  effects on leaf-level and whole-plant level WUE. I found that at leaf-level, WUE of both C3 and C4 plants responded in proportion to the increase in  $C_a$ , but that in C3 plants the change in WUE was due to both changes in assimilation and transpiration whereas in C4 plants the change in WUE was primarily due to the reduction in transpiration. At whole plant level, the WUE response was less than proportional to the  $C_a$  increase, as predicted. The discrepancy was larger in C4 (only 70 – 79% of the  $C_a$  increase) than in C3 plants (80 – 99%). The reduction occurred because whole-plant transpiration was less sensitive to  $C_a$  than leaf transpiration, whereas whole-plant biomass gains were similar in size to photosynthetic responses. This work informs models by analysing the effects of  $eC_a$  on WUE in terms that can be directly compared against model predictions.



# CHAPTER 1

## 1.1 Introduction

Anthropogenic emissions of ‘greenhouse gases’ are causing major changes to global climate (IPCC 2013). Of these greenhouse gases, carbon dioxide (CO<sub>2</sub>) is the most significant. CO<sub>2</sub> is also the essential substrate of photosynthesis, the primary biological process which drives plant growth (Sharkey 1985, Long and Drake 1992, Bowes 1993). To predict the potential impact of climate change on plant growth, mathematical models based on plant physiological processes are used. These models are sets of mathematical equations which represent our knowledge or hypotheses about working of ecosystems and their responses to the environment. For accurate predictions, it is important that these models are up to date and reflect current empirical knowledge of plant physiological processes.

During the last few decades, thousands of experiments have been performed on CO<sub>2</sub> effects on plants (Körner 2006). Data from these experiments have not been fully exploited in their use in the current generation of models. The current models have failed to keep pace with and integrate new information reported by these experiments (For example models listed in De Kauwe et al. 2013, Zaehle et al. 2014). The models do not incorporate current empirical knowledge about long-term CO<sub>2</sub> responses and variability among species and ecosystem types. This disconnect between models and experiments occurs mainly because experimental data are rarely reported into forms that can be used directly into models. The present research therefore aims to examine data in ways that can be used to form links between existing experimental data with the models. The approach taken was to synthesise experimental results from previous studies and from newly targeted experiments into quantitative expressions that can be incorporated into models.

This chapter provides an overall introduction to the thesis. The content is divided into two main sections.

- I. A summary of models used in the thesis.
- II. A summary of model-oriented questions addressed in the thesis.

The first section gives a synopsis of the models which were considered in the present research. The models explained are ‘C<sub>3</sub> photosynthesis model’ and ‘C<sub>3</sub> stomatal conductance models’. Additionally, plant ‘Water use efficiency’ is explained in context since it is

measured as a ratio of photosynthesis to water loss through transpiration. This is followed by ‘C4 photosynthesis model’ and ‘C4 stomatal conductance model’. The second section gives an overview of the model-oriented questions addressed in the thesis.

## SECTION I

### 1.2 C3 photosynthesis model

C3 plants, which constitute more than 90% of terrestrial plant species (Long et al. 2004), are called C3 because the first product of carboxylation (fixing of CO<sub>2</sub>) is a 3-carbon acid, phosphoglyceric acid (PGA) in the first step of the Calvin-Benson cycle. For summarizing and synthesizing the contributing mechanisms involved in C3 photosynthesis, the biochemical model published by Farquhar et al. and Farquhar & von Caemmerer (1980, 1982) is widely used. The model makes use of a few key physiological processes, and can be incorporated into models analysing C3 biochemistry at leaf-level (von Caemmerer and Farquhar 1981) through to predicting photosynthetic fluxes at ecosystem-level in response to global environmental change (Wang and Jarvis 1990, Long 1991, Amthor 1995, Lloyd and Farquhar 1996, De Pury and Farquhar 1997, Sellers et al. 1997, Field and Avissar 1998, Lloyd 1999).

#### *Limitations assumed in the model*

By integrating various aspects of the biochemistry of C3 photosynthesis, the Farquhar et al. and Farquhar & von Caemmerer (1980, 1982) model is formulated in a way that allows easy interpretation of leaf gas exchange studies. In the model, the biochemical reactions of photosynthesis are considered to be in one of two distinct limitation states.

In first limitation, the reaction is limited by the enzyme Rubisco (ribulose 1-5 bisphosphate carboxylase/oxygenase). At current C<sub>a</sub> and light saturating conditions, Rubisco is supposed to limit the photosynthetic rate, denoted as A<sub>c</sub>. A<sub>c</sub> is calculated as a function of the maximum carboxylation capacity of Rubisco (V<sub>cmax</sub>) by:

$$A_c = \frac{(C_i - \Gamma^*)V_{cmax}}{C_i + K_c(1 + O/K_o)} - R_d \quad (1)$$

where C<sub>i</sub> is the intercellular CO<sub>2</sub> concentration, K<sub>c</sub> and K<sub>o</sub> are Michaelis–Menten constants of Rubisco for CO<sub>2</sub> and O<sub>2</sub>, respectively, and Γ\* is the CO<sub>2</sub> compensation point in the absence of day respiration (R<sub>d</sub>). Because of its dependence on maximum Rubisco activity, V<sub>cmax</sub>, A<sub>c</sub> is also often called the Rubisco-limited rate of CO<sub>2</sub> assimilation.

The second limitation is the capacity of the thylakoid reactions (inside chloroplast) to regenerate RuBP. The capacity of RuBP regeneration reflects the rate of electron transport. Under the conditions of greater than current  $C_a$ , or under low-light conditions, RuBP regeneration or electron-transport capacity usually limits photosynthesis. This limitation is denoted as  $A_j$ .  $A_j$  is calculated as

$$A_j = \frac{(C - \Gamma_*)J}{4C + 8\Gamma_*} - R_d \quad (2)$$

Where  $J$  is the rate of electron transport at a given irradiance.

In the first rate limiting step, the enzyme Rubisco has the affinity to react with both  $CO_2$  and  $O_2$ . In the reaction with  $CO_2$  (carboxylation), photosynthesis occurs via carbon reduction cycle, whereas in the reaction with  $O_2$  (oxygenation), photorespiration occurs via photosynthetic carbon oxidation. High temperature favours the oxygenation reaction while elevated  $CO_2$  concentration ( $eC_a$ ) inhibits oxygenation and increases photosynthesis. Thus, the inhibition of oxygenation by rising  $C_a$  will have the largest effect at higher temperatures. Hence, at the leaf scale, as shown by leaf-level model predictions by Long (1991), an interactive effect is expected between rising  $C_a$  and temperature. Many models of the response of vegetation to climate change take into account this  $eC_a \times$  temperature interaction effect on leaf photosynthesis while some models do not incorporate this important interaction (Medlyn et al. 2011b). Chapter 2 of this thesis asks the question whether the present data from  $eC_a$  and temperature studies support a  $eC_a \times$  temperature interaction as predicted by this model.

### 1.3 C3 stomatal conductance models

Exchange of  $CO_2$  and water vapour occurs through stomates- small apertures on the leaves of the plants. By opening to acquire  $CO_2$  as photosynthetic substrate whilst still maintaining favourable  $H_2O$  balance in leaf tissues, stomates of plants facilitate a key plant physiological trade-off (Cowan 1982, Zeiger 1987). To understand and predict the  $CO_2$  effect on stomatal conductance, numerous models have been designed to analyse and synthesise the complex behaviour of stomatal opening. The models range in complexity from the simple empirical to the highly detailed mechanistic.

#### 1.3.1 Empirical models

Empirical or phenomenological models are based on observed responses to environmental factors and on a reference value specific to a species or functional type. The well-known and simplest stomatal conductance ( $g_s$ ) model is the experimental model of Jarvis

(1976). This model is derived from experimentally determined relations between  $g_s$  and environmental variables; quantum flux density (Q), ambient  $\text{CO}_2$  concentration ( $C_a$ ), vapour pressure deficit (D), temperature (T) and leaf/soil water potential ( $\Psi$ ). The underlying assumption of the model is that the influence of each environmental factor on  $g_s$  is independent of the others and can be determined by boundary line analysis (Webb 1972). The Jarvis model (1976), in its first form, integrates the responses of  $g_s$  to light intensity, leaf temperature, vapour pressure deficit, ambient  $C_a$  and leaf water potential, according to the following equation:

$$g_s = f(Q).f(T).f(D).f(\Psi).f(C_a) \quad (3)$$

where Q is the quantum flux density ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), T is the leaf temperature ( $^{\circ}\text{C}$ ), D is the leaf-to-air vapour pressure deficit calculated at leaf temperature (kPa),  $C_a$  is the ambient  $\text{CO}_2$  concentration ( $\mu\text{mol mol}^{-1}$ ) and  $\Psi$  is leaf water potential (MPa).

The Jarvis model is diagnostic and does not include feedback loops between  $g_s$ , internal  $\text{CO}_2$ , transpiration, humidity deficits and leaf water potential (Farquhar 1978, Jones and Rawson 1979, Jones 1992).

### 1.3.2 Coupled photosynthesis-stomatal conductance models

Developments in eco-physiological concepts led to alternative approaches for calculating stomatal conductance. In the 1970's, Wong et al. (1979) demonstrated that stomatal conductance was tightly coupled to leaf photosynthesis. From the observations of Wong et al. (1979) and their own laboratory experiments, Ball et al. (1987) and Collatz et al. (1991) also published a model (known as the Ball-Berry model) that linked stomatal conductance to leaf photosynthesis, humidity deficit and  $\text{CO}_2$  concentration at the leaf surface ( $C_s$ ):

$$g_s = m \frac{A \text{ RH}}{C_s} + g_o \quad (4)$$

where m is a coefficient representing a dimensionless slope, RH is relative humidity at the leaf surface,  $g_o$  is the zero intercept, and A ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is leaf photosynthesis. Typically, A is derived from a biochemical model of photosynthesis of Farquhar and von Caemmerer (1982). A third equation describing diffusion of  $\text{CO}_2$  through stomata,

$$g_s = \frac{A}{C_s \left[ 1 - \frac{C_i}{C_s} \right]} \quad (5)$$

allows the three linked variables A,  $g_s$  and  $C_s$  to be calculated.



### 1.3.3 Optimal model

Cowan and co-workers (Cowan 1977, Cowan and Farquhar 1977) developed a theory for stomatal behaviour based on the principle that gas exchange through stomata is optimal when the maximal amount of carbon is assimilated (A) for a given amount of water lost (E). Optimal stomatal behaviour was defined as the state which maintained

$$\frac{\partial E}{\partial A} = \text{constant (or } = \lambda) \quad (6)$$

Cowan used economic theory to explain this relationship, with the slope,  $\partial E/\partial A$ , describing the marginal cost. According to the theory plants tend to maintain a uniform marginal cost of water lost and the benefit per unit carbon gained, but the theory fails to predict the value of  $\lambda$ . The theory however, led to a new generation of stomatal conductance models based on optimisation principles rather than on empirical values. Model implementations of this theory have been attempted (Hari et al. 1986, Lloyd 1991, Arneth et al. 2002, Katul et al. 2010), but several issues have restricted wider use of these implementations. A key problem has been parameterization of  $\lambda$ , which is perceived as difficult to estimate.

### 1.3.4 Combined empirical and optimal stomatal conductance model

Recently, Medlyn et al. (2011a) proposed an optimal stomatal model that combines the optimal theory and empirically fit parameters based on experimental observations of stomatal behaviour in response to environmental conditions. The ‘unified optimal stomatal model’ by Medlyn et al. (2011a) is similar to the empirical stomatal models developed by Collatz et al. (1991) and Leuning (1995) but provides a theoretical interpretation for model parameter values. The unified optimal stomatal model has the form:

$$g_s = g_0 + 1.6(1 + \frac{g_1}{\sqrt{D}}) \frac{A}{c_a} \quad (7)$$

where D is the leaf to air vapour pressure deficit (kPa),  $g_0$  is the cuticular conductance and the slope  $g_1$  is a parameter which is described to be inversely proportional to the marginal cost of water to the plant  $\lambda$  (Medlyn et al. 2011a) i.e.

$$g_1 \propto \sqrt{\frac{\Gamma^*}{\lambda}} \quad (8)$$

The unified model offers a new and simple means of quantifying  $\lambda$  by fitting equation 7 to  $g_s$  measurements and using the fitted parameter  $g_1$  as a proxy to calculate  $\lambda$ .  $\Gamma^*$  is not known for all C3 species but is used as one reference value for all C3 species.

## 1.4 Water use efficiency

Plants affect hydrological and carbon cycles, directly with their water use efficiency (WUE) which is defined as the ratio of CO<sub>2</sub> gain by assimilation,  $A$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), to water loss by transpiration,  $E$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ):

$$\text{WUE} = \frac{A}{E} \quad (9)$$

Plant water use efficiency is also sometimes defined as  $A/g_s$  or, at whole plant scale, by biomass increment/ $E$ . In this thesis, plant water use efficiency has been referred to each of these terms as: intrinsic water use efficiency ( $A/g_s = i\text{WUE}$ ,  $\text{mmolCO}_2 (\text{molH}_2\text{O})^{-1}$ ), instantaneous transpiration efficiency ( $A/E = i\text{TE}$ ,  $\text{mmolCO}_2 (\text{molH}_2\text{O})^{-1}$ ) and whole plant water use efficiency ( $\text{Biomass} / E = \text{WUE}$ ,  $\text{g DM (kg H}_2\text{O)}^{-1}$ ).

The Ball-Berry (Ball et al. 1987) and Medlyn et al. (2011a) models both imply that water use efficiency should be proportional to increasing  $C_a$ . Rearranging Equation (7) it can be shown that if  $g_0$  is close to zero, therefore:

$$\frac{A}{g_s} \cong \frac{C_a}{1.6} \left(1 + \frac{g_1}{\sqrt{D}}\right) \quad (10)$$

If  $D$  is constant, this implies that  $A/g_s = i\text{WUE}$  is proportional to  $C_a$ . If the leaf is well-coupled to the surrounding air (leaf boundary layer conductance is high) then transpiration,  $E$ , is proportional to  $g_s$ , i.e.

$$E = \frac{g_s D}{P} \quad (11)$$

where  $P$  is the atmospheric pressure (kPa).

This means that  $i\text{TE}$ ,  $A/E$ , is also proportional to  $C_a$  when  $D$  is constant. This equation implies that a doubling of  $C_a$  will increase plant water use efficiency two fold. However, this equation applies at the leaf level and only when stomata strongly control transpiration rates, that is, when the leaf is ‘well-coupled’ to the atmosphere (Jarvis and Mcnaughton 1986).

At canopy scale, photosynthesis per unit transpiration is less than proportional to  $C_a$  because transpiration is not directly proportional to canopy conductance. The control of transpiration by  $g_s$  depends on the coupling of the canopy to the air above, which is related to the boundary layer effect. Leaf boundary layer is the air immediate to the stomatal opening and is assumed to be motionless. The subsequent layers above boundary layer have turbulent air movements which remove water vapour more efficiently. If the air around leaf is in high motion, it is termed “well-coupled” and there is strong stomatal control of transpiration (Jarvis and Mcnaughton 1986). Shorter canopies such as of crops and herbs, show poorer aerodynamic coupling of vegetation to the atmospheric surface layer, and therefore less

stomatal control of transpiration. In comparison, tall forest canopies experience a more turbulent and faster air-stream and a greater coupling. This results in forest canopy leaves being well-coupled to the atmosphere around them. Models incorporate these vegetation-atmosphere feedback processes that affect responses of evapotranspiration to changes in stomatal conductance (Mcnaughton and Jarvis 1991, Jacobs and de Bruin 1997, Wilson et al. 1999, Grant et al. 2001, De Kauwe et al. 2013). However, models disagree about how strongly coupled plants are to the atmosphere, and therefore they predict different  $\text{CO}_2$  effects on canopy water use efficiency. De Kauwe et al. (2013) showed that some models assume high coupling and predict that canopy-scale water use efficiency is almost proportional to  $\text{CO}_2$  whereas other models assume poor coupling and predict that water use efficiency is relatively unaffected by  $\text{CO}_2$ . This is the basis of the question addressed in my fourth chapter: do experimental data support the prediction that WUE is proportional to  $\text{CO}_2$  and, if so, at what scales?

## 1.5 C4 photosynthesis model

C4 term refers to the different pathway that some plants use to capture carbon dioxide during photosynthesis. The C4 pathway evolved from the primitive C3 pathway in species in the wet and dry tropics during late Miocene and Pliocene (3 to 8 million years ago) (Edwards et al. 2010). In C3 photosynthesis, 3-phosphoglyceric acid (3-PGA) is the first product formed in the fixation of  $\text{CO}_2$ , and the enzyme Rubisco catalyses the reaction. In C4 photosynthesis, atmospheric  $\text{CO}_2$  is first fixed into C4 acids in mesophyll cells by the enzyme PEPC (phosphoenolpyruvate carboxylase) and C4 acids donate  $\text{CO}_2$  to enzyme Rubisco in the bundle sheath cells.

The mathematical model for the C4 pathway, formulated by von Caemmerer (2000), is based on those of Berry and Farquhar (1978) and Peisker (Peisker 1979). In the model, properties of the two enzymes have been combined which work in two compartments, PEPC in mesophyll cells which has a higher affinity for atmospheric  $\text{CO}_2$  and Rubisco in bundle sheath cells which has a lower affinity for  $\text{CO}_2$  plus sensitivity to  $\text{O}_2$ .

### *Limitations assumed in the model*

$\text{CO}_2$  transfer from intercellular air spaces to mesophyll cells may be large enough in C4 leaves (Pfeffer and Peisker 1998). However, the bundle-sheath conductance ( $g_{bs}$ ) is a major factor that determines the rate of  $\text{CO}_2$  leakage from the bundle sheath to the mesophyll

(L). Since bundle-sheath compartment is semi-enclosed and it relies on mesophyll cells for the supply of CO<sub>2</sub> in the form of C4 acids, the CO<sub>2</sub> assimilation rate, A, can be written in terms of the mesophyll reactions as:

$$A = V_p - L - R_m \quad (12)$$

where V<sub>p</sub> is the rate of PEP carboxylation, R<sub>m</sub> is the mitochondrial respiration occurring in the mesophyll and L is the rate of CO<sub>2</sub> leakage from the bundle sheath to the mesophyll. The leakage, L, is given by:

$$L = g_{bs}(C_s - C_m) \quad (13)$$

where g<sub>bs</sub> is the physical conductance to CO<sub>2</sub> leakage and is determined by the properties of the bundle-sheath cell wall; C<sub>s</sub> and C<sub>m</sub> are the bundle-sheath and mesophyll CO<sub>2</sub> partial pressures. The CO<sub>2</sub> leakage depends upon the equilibrium rates of PEP carboxylation and Rubisco activity and the conductance of the bundle sheath to CO<sub>2</sub>. Leakiness (Φ) defines leakage as a fraction of the rate of PEP carboxylation and thus describes the efficiency of the C4 cycle:

$$\Phi = L/V_p \quad (14)$$

The additional limitation to C4 cycle is the rate of PEP carboxylation. When CO<sub>2</sub> is limiting, the rate is given by a Michaelis–Menten equation:

$$V_p = \frac{C_m V_{pmax}}{C_m + K_p} \quad (15)$$

where V<sub>pmax</sub> is the maximum PEP carboxylation rate and K<sub>p</sub> is the Michaelis–Menten constant for CO<sub>2</sub>. This assumes that the substrate PEP is saturating under these conditions.

Similar to C3 model of photosynthesis (Farquhar et al. 1980, Farquhar and Caemmerer 1982) Rubisco carboxylation rate at high light intensity is given by its RuBP-saturated rate:

$$V_c = \frac{C_s V_{cmax}}{C_s + K_c(1 + O_s/K_o)} \quad (16)$$

where O<sub>s</sub> is the O<sub>2</sub> partial pressure in the bundle sheath.

The Rubisco-limited rate of CO<sub>2</sub> assimilation is given by:

$$A_c = \frac{(C_s - \gamma^* O_s) V_{cmax}}{C_s + K_c(1 + O_s/K_o)} - R_d \quad (17)$$

where O<sub>s</sub> is the O<sub>2</sub> partial pressure in the bundle sheath and  $\gamma^* = 0.5[V_{oamax} K_c / (V_{cmax} K_o)]$

The term  $\gamma$  is highlighted since O<sub>2</sub> partial pressure in bundle sheath may vary.

The RuBP-regeneration reaction A<sub>j</sub> is given by:

$$A_j = \frac{(1 - \gamma^* O_s / C_s)(1 - x) J_t}{3(1 + 7\gamma^* O_s / (3C_s))} - R_d \quad (18)$$

Both equations of electron transport-limited reaction for C3 and C4 are similar.

## 1.6 C4 stomatal conductance model

Collatz et al. (1992) proposed a linear model of  $A$  and  $g_s$  for C4 photosynthetic pathway. They combined leaf surface  $CO_2$  level ( $C_s$ ) and relative humidity (RH) with the C4 photosynthesis model to give leaf photosynthesis as a function of absorbed quantum flux,  $CO_2$ , temperature,  $C_s$ , and RH levels.

To date no recent attempt has been made to model the  $CO_2$  response of  $g_s$  in C4 species or to ask whether the response to  $CO_2$  can be predicted from the equation (4). Optimal stomatal behaviour has been predicted for C3 species (Manzoni et al. 2011, Medlyn et al. 2011a) but has not been tested for  $CO_2$  responses of C4  $g_s$ . Optimal stomatal theory may predict different responses for C3 than C4 photosynthesis due to different limitations assumed in both models. In my third chapter I used optimal stomatal theory to predict C4 stomatal responses and test whether these are supported by data or not.

## SECTION II

## 1.7 Summary of research hypotheses

The main aim of the study was to link current experimental findings to that of model predictions. I addressed several aspects of modeling  $CO_2$  responses of vegetation and formulated the following research hypotheses.

### **Chapter 2: Does the growth response of woody plants to elevated $CO_2$ increase with temperature? A model-oriented meta-analysis**

*Hypothesis: Due to the kinetics of the enzyme Rubisco, tree responses to elevated  $CO_2$  should be greater at higher growth temperature and consequently the  $CO_2$  response of NPP should be greater in tropical as compared to boreal climate ecosystems.*

The temperature dependence of the reaction kinetics of the enzyme Rubisco implies that the response of photosynthesis to rising  $C_a$  will be greater with increasing temperature. Vegetation models incorporating this interaction predict that the response of plant and ecosystem net primary productivity (NPP) to elevated  $CO_2$  increases with rising temperature, and will be substantially larger in warm tropical forests than in cold boreal forests. It is unclear whether experimental data support these predictions.

To test the above hypothesis, I used the meta-analysis approach to test specifically whether empirical data support the assumption of a positive interaction between  $CO_2$  and

temperature that is embedded in some vegetation models. I carried out two meta-analyses. In the first meta-analysis, I examined factorial CO<sub>2</sub> x temperature experiments to test for an interaction-term between the CO<sub>2</sub> and temperature treatments. In the second meta-analysis, I examined field-based experiments across the globe to test the model-based hypothesis that the elevated CO<sub>2</sub> effect on plant biomass increases with mean annual temperature.

### **Chapter 3: Do C4 plants exhibit optimal stomatal behaviour? A test with congeneric C3 and C4 species**

*Hypotheses: (a) The optimal stomatal model by Medlyn et al. (2011a) can be used for C4 plants to predict their stomatal behaviour. (b) Stomata of C4 plants are more sensitive to rising CO<sub>2</sub> concentrations than those of C3 plants.*

Optimal stomatal theory says that stomata should act to maximize carbon gain (photosynthesis,  $A$ ) while minimizing water loss (transpiration,  $E$ ). That is, the optimal stomatal behaviour is to maximise the integrated sum of  $(A - \lambda E)$ , where  $\lambda$  ( $\text{mol C mol}^{-1} \text{H}_2\text{O}$ ) represents the marginal carbon cost of water use. The unified stomatal conductance model by Medlyn et al. (2011a) captures stomatal responses for the C3 plants. Since C4 plants have different photosynthetic pathway and saturate at lower CO<sub>2</sub> levels, the optimal stomatal behaviour for C4 plants should differ from that of C3 plants. I used optimal stomatal theory to predict the optimal stomatal behaviour of C4 plants. The theory predicts that stomatal conductance of C4 plants should follow the Medlyn et al. (2011a) stomatal model but with lower  $g_1$  values, and that stomata of C4 plants should be more sensitive to increasing  $C_a$  than C3 plants.

A glasshouse-based elevated CO<sub>2</sub> experiment was designed to specifically address the second hypothesis of this section of my thesis. Closely related C3 and C4 species were selected for the study and were grown in ambient CO<sub>2</sub> glasshouse for four months. Stomatal conductance responses to varying  $C_a$  and VPD were measured to compare the experimental outcome with the model predictions.

### **Chapter 4: Is plant water use efficiency proportional to atmospheric CO<sub>2</sub>?**

*Hypotheses: (a) Water use efficiency of plants increases in proportion to increase in CO<sub>2</sub> both at leaf and whole-plant scales. (b) The response of WUE to elevated CO<sub>2</sub> differs among plant functional types.*

Optimal stomatal theory predicts that leaf-level water use efficiency (WUE) of plants should be proportional to CO<sub>2</sub>. However, whole-plant WUE is predicted to be somewhat less

responsive than leaf-level WUE due to boundary layer effects on canopy transpiration. Models predict A/E should be proportional to  $C_a$  in all plant species. According to their different sensitivity of photosynthesis and  $g_s$ , it was hypothesized that WUE of C4 plants would be less responsive to  $CO_2$  than that of C3 plants; and that WUE among C3 plants would respond in this order: C3-herbs/crops > angiosperm trees > gymnosperm trees.

To assess how well the existing literature supports model predictions, I carried out meta-analysis by extracting data from the literature and statistically combining these studies on increased  $CO_2$  effects on leaf-level and whole-plant level WUE.

## Chapter 5: Discussion and conclusions

The overall aim of the thesis was to use experimental data to test and inform current models of  $CO_2$  responses. The conclusions for modelling from my research are described in Chapter 5.

## References

- Amthor, J. S. 1995. Terrestrial Higher-Plant Response to Increasing Atmospheric [ $CO_2$ ] in Relation to the Global Carbon-Cycle. *Global Change Biology* 1:243-274.
- Arneth, A., J. Lloyd, H. Santruckova, M. Bird, S. Grigoryev, Y. N. Kalaschnikov, G. Gleixner, and E. D. Schulze. 2002. Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric  $CO_2$  concentration. *Global Biogeochemical Cycles* 16.
- Ball, J. T., I. Woodrow, and J. Berry. 1987. A Model Predicting Stomatal Conductance and its Contribution to the Control of Photosynthesis under Different Environmental Conditions. Pages 221-224 in J. Biggins, editor. *Progress in Photosynthesis Research*. Springer Netherlands.
- Berry, J. A. and G. D. Farquhar. 1978. The  $CO_2$  concentrating function of C4 photosynthesis. A biochemical model. Pages 119-131 in *Proc. of the 4th International Congress on Photosynthesis*. London, The Biochemical Soc., Reading, England.
- Bowes, G. 1993. Facing the Inevitable - Plants and Increasing Atmospheric  $CO_2$ . *Annual Review of Plant Physiology and Plant Molecular Biology* 44:309-332.
- Collatz, G. J., J. T. Ball, C. Grivet, and J. A. Berry. 1991. Physiological and Environmental-Regulation of Stomatal Conductance, Photosynthesis and Transpiration - a Model That Includes a Laminar Boundary-Layer. *Agricultural and Forest Meteorology* 54:107-136.
- Collatz, G. J., M. Ribas-Carbo, and J. A. Berry. 1992. Coupled Photosynthesis-Stomatal Conductance Model for Leaves of C4 Plants. *Australian Journal of Plant Physiology* 19:519-538.
- Cowan, I. R. 1977. Stomatal behaviour and environment. *Adv. Bot. Res.* 4:117-228.
- Cowan, I. R. 1982. Regulation of water use in relation to carbon gain in higher plants. Pages 589-613.
- Cowan, I. R. and G. D. Farquhar. 1977. Stomatal function in relation to leaf metabolism and environment. *Symp Soc Exp Biol* 31:471-505.
- De Kauwe, M. G., B. E. Medlyn, S. Zaehle, A. P. Walker, M. C. Dietze, T. Hickler, A. K. Jain, Y. Q. Luo, W. J. Parton, I. C. Prentice, B. Smith, P. E. Thornton, S. S. Wang, Y. P. Wang, D. Warland, E. S. Weng, K. Y. Crous, D. S. Ellsworth, P. J. Hanson, H. Seok Kim, J. M. Warren, R. Oren, and R. J. Norby. 2013. Forest water use and water use efficiency at elevated  $CO_2$ : a model-data intercomparison at two contrasting temperate forest FACE sites. *Global Change Biology* 19:1759-1779.

- De Pury, D. G. G. and G. D. Farquhar. 1997. Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant, Cell & Environment* 20:537-557.
- Edwards, E. J., C. P. Osborne, C. A. E. Strömberg, S. A. Smith, and C. G. Consortium. 2010. The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science* 328:587-591.
- Farquhar, G. D. 1978. Feedforward Responses of Stomata to Humidity. *Aust. J. Plant Physiol.* 5:787-800.
- Farquhar, G. D. and S. Caemmerer. 1982. Modelling of Photosynthetic Response to Environmental Conditions. Pages 549-587 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. *Physiological Plant Ecology II*. Springer Berlin Heidelberg.
- Farquhar, G. D., S. Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta* 149:78-90.
- Field, C. B. and R. Avissar. 1998. Bidirectional interactions between the biosphere and the atmosphere - Introduction. *Global Change Biology* 4:459-460.
- Grant, R. F., B. A. Kimball, T. J. Brooks, G. W. Wall, P. J. Pinter, D. J. Hunsaker, F. J. Adamsen, R. L. Lamorte, S. W. Leavitt, T. L. Thompson, and A. D. Matthias. 2001. Modeling Interactions among Carbon Dioxide, Nitrogen, and Climate on Energy Exchange of Wheat in a Free Air Carbon Dioxide Experiment. *Agron. J.* 93:638-649.
- Hari, P., A. Makela, E. Korpilahti, and M. Holmberg. 1986. Optimal control of gas exchange. *Tree Physiology* 2:169-175.
- IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jacobs, C. M. J. and H. A. R. de Bruin. 1997. Predicting Regional Transpiration at Elevated Atmospheric CO<sub>2</sub>: Influence of the PBL-Vegetation Interaction. *Journal of Applied Meteorology* 36:1663-1675.
- Jarvis, P. G. 1976. The Interpretation of the Variations in Leaf Water Potential and Stomatal Conductance Found in Canopies in the Field. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 273:593-610.
- Jarvis, P. G. and K. G. Mcnaughton. 1986. Stomatal Control of Transpiration - Scaling up from Leaf to Region. *Advances in Ecological Research* 15:1-49.
- Jones, H. G. 1992. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge University Press.
- Jones, M. M. and H. M. Rawson. 1979. Influence of rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency, and osmotic potential in sorghum. *Physiol. Plant.* 45:103-111.
- Katul, G., S. Manzoni, S. Palmroth, and R. Oren. 2010. A stomatal optimization theory to describe the effects of atmospheric CO<sub>2</sub> on leaf photosynthesis and transpiration. *Annals of Botany* 105:431-442.
- Körner, C. 2006. Plant CO<sub>2</sub> responses: an issue of definition, time and resource supply. *New Phytologist* 172:393-411.
- Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. *Plant, Cell & Environment* 18:339-355.
- Lloyd, J. 1991. Modeling Stomatal Responses to Environment in *Macadamia-Integrifolia*. *Australian Journal of Plant Physiology* 18:649-660.
- Lloyd, J. 1999. The CO<sub>2</sub> dependence of photosynthesis, plant growth responses to elevated CO<sub>2</sub> concentrations and their interaction with soil nutrient status, II. Temperate and boreal forest productivity and the combined effects of increasing CO<sub>2</sub> concentrations and increased nitrogen deposition at a global scale. *Functional Ecology* 13:439-459.
- Lloyd, J. and G. D. Farquhar. 1996. The CO<sub>2</sub> dependence of photosynthesis, plant growth responses to elevated atmospheric CO<sub>2</sub> concentrations and their interaction with soil nutrient status .1. General principles and forest ecosystems. *Functional Ecology* 10:4-32.
- Long, S. P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO<sub>2</sub> concentrations: Has its importance been underestimated? *Plant, Cell & Environment* 14:729-739.



- Long, S. P., E. A. Ainsworth, A. Rogers, and D. R. Ort. 2004. Rising atmospheric carbon dioxide: Plants face the future. *Annual Review of Plant Biology* 55:591-628.
- Long, S. P. and B. G. Drake. 1992. Photosynthetic CO<sub>2</sub> assimilation and rising atmospheric CO<sub>2</sub> concentrations. Pages 69-103 in N. R. B. a. H. Thomas., editor. *Crop Photosynthesis: Spatial and Temporal Determinants*. Elsevier Science Publishers B.V., Amsterdam.
- Manzoni, S., G. Vico, G. Katul, P. A. Fay, W. Polley, S. Palmroth, and A. Porporato. 2011. Optimizing stomatal conductance for maximum carbon gain under water stress: a meta-analysis across plant functional types and climates. *Functional Ecology* 25:456-467.
- Mcnaughton, K. G. and P. G. Jarvis. 1991. Effects of Spatial Scale on Stomatal Control of Transpiration. *Agricultural and Forest Meteorology* 54:279-302.
- Medlyn, B. E., R. A. Duursma, D. Eamus, D. S. Ellsworth, I. C. Prentice, C. V. M. Barton, K. Y. Crous, P. de Angelis, M. Freeman, and L. Wingate. 2011a. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17:2134-2144.
- Medlyn, B. E., R. A. Duursma, and M. J. B. Zeppel. 2011b. Forest productivity under climate change: a checklist for evaluating model studies. *Wiley Interdisciplinary Reviews-Climate Change* 2:332-355.
- Peisker, M. 1979. Conditions for low, and oxygen independent CO<sub>2</sub> compensations concentrations in C4 plants as derived from a simple model. *Photosynthetica* 13:198-207.
- Pfeffer, M. and M. Peisker. 1998. CO<sub>2</sub> gas exchange and phosphoenolpyruvate carboxylase activity in leaves of *Zea mays* L. *Photosynthesis Research* 58:281-291.
- Sellers, P. J., R. E. Dickinson, D. A. Randall, A. K. Betts, F. G. Hall, J. A. Berry, G. J. Collatz, A. S. Denning, H. A. Mooney, C. A. Nobre, N. Sato, C. B. Field, and A. Henderson-Sellers. 1997. Modeling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science* 275:502-509.
- Sharkey, T. D. 1985. Photosynthesis in Intact Leaves of C-3 Plants - Physics, Physiology and Rate Limitations. *Botanical Review* 51:53-105.
- von Caemmerer, S. 2000. Biochemical models of leaf photosynthesis / S. von Caemmerer. CSIRO Publishing, Collingwood, Vic.
- von Caemmerer, S. and G. D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376-387.
- Wang, Y. P. and P. G. Jarvis. 1990. Influence of Crown Structural-Properties on Par Absorption, Photosynthesis, and Transpiration in Sitka Spruce - Application of a Model (Maestro). *Tree Physiology* 7:297-316.
- Webb, R. A. 1972. Use of the boudary line in the analysis of biological data. *J Hort Sci* 47:309-319.
- Wilson, K. B., T. N. Carlson, and J. A. Bunce. 1999. Feedback significantly influences the simulated effect of CO<sub>2</sub> on seasonal evapotranspiration from two agricultural species. *Global Change Biology* 5:903-917.
- Wong, S. C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424-426.
- Zaehle, S., B. E. Medlyn, M. G. De Kauwe, A. P. Walker, M. C. Dietze, T. Hickler, Y. Luo, Y. -P. Wang, B. El-Masri, P. Thornton, A. Jain, S. Wang, D. Warlind, E. Weng, W. Parton, C. M. Iversen, A. Gallet-Budynek, H. McCarthy, A. Finzi, P. J. Hanson, I. C. Prentice, R. Oren, and R. J. Norby. 2014. Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO<sub>2</sub> Enrichment studies. *New Phytologist* 202:803-822.
- Zeiger, E., Farquhar, G. D., & Cowan, I. R. . 1987. Stomatal Function. Stanford University Press, Stanford, California.

## CHAPTER 2

### **Does the growth response of woody plants to elevated CO<sub>2</sub> increase with temperature? A model-oriented meta-analysis**

**Summary** The temperature dependence of the reaction kinetics of the enzyme Rubisco implies that - at the level of a chloroplast - the response of photosynthesis to rising atmospheric CO<sub>2</sub> (C<sub>a</sub>) will increase with increasing air temperature. Vegetation models incorporating this interaction predict that the response of plant and ecosystem net primary productivity (NPP) to elevated CO<sub>2</sub> (eC<sub>a</sub>) increases with rising temperature, and be substantially larger in warm tropical forests than in cold boreal forests. It is unclear whether experimental data support these predictions. I specifically tested these model predictions against evidence from eC<sub>a</sub> experiments by carrying out two meta-analyses. Firstly, I tested for an interaction effect on growth responses in factorial eC<sub>a</sub> x temperature experiments. This analysis showed a positive, but non-significant interaction effect (8.2% average, CI (-0.85, 17.99) between eC<sub>a</sub> and temperature. Secondly, I examined eC<sub>a</sub> experiments on woody plants across the globe to test for a relationship between the eC<sub>a</sub> effects and mean annual temperature (MAT). This second analysis also showed a positive but non-significant correlation between the eC<sub>a</sub> effect on plant biomass responses and MAT. The magnitude of the interactions between C<sub>a</sub> and temperature found in both meta-analyses were consistent with model predictions, even though both analyses gave non-significant results. Thus, it was not possible to distinguish between the competing hypotheses of no interaction versus an interaction based on Rubisco kinetics from the available experimental database. Experiments in a wider range of temperature zones are required. Until such experimental data are available, model predictions should aim to incorporate this uncertainty.

### **2.1 Introduction**

Increasing levels of carbon dioxide in the atmosphere due to anthropogenic activities are likely to increase mean global temperatures by about 2 - 5°C during the next century, with concomitant changes in other environmental variables such as rainfall patterns and humidity (IPCC 2013). These changes will impact on forest productivity in a number of ways. Some responses are likely to be positive, such as enhancement of photosynthetic rates by rising atmospheric CO<sub>2</sub> (Ainsworth and Long 2005, Hyvonen et al. 2007, Kirschbaum 2011), and

extension of growing seasons by warmer temperatures (Norby et al. 2003, Linderholm 2006, Taylor et al. 2008), whilst others may be negative, such as increasing drought impacts due to higher evaporative demand and reduced rainfall (Knapp et al. 2002, Barnett et al. 2005, IPCC 2007). To predict the overall impact of climate change on tree growth, we rely on mathematical models that have been developed based on our understanding of environmental influences on plant physiological processes (Medlyn et al. 2011, Reyer et al. 2014). Such models of forest response to climate change are essential for many purposes, including management of forest lands (Mäkelä et al. 2000, Canadell and Raupach 2008) and prediction of the terrestrial carbon cycle (Sitch et al. 2008, Lewis et al. 2013). It is therefore important to ensure that the assumptions made by such models are strongly underpinned by scientific understanding and empirical data.

One important assumption made in many models is that there is a positive interaction between  $eC_a$  and temperature ( $T$ ) on photosynthesis. At the biochemical level in  $C_3$  plants,  $eC_a$  stimulates photosynthesis by increasing the rate of the carboxylation reaction relative to the oxygenation reaction in the photosynthetic carbon reduction cycle. In contrast, an increase in  $T$  increases the rate of oxygenation relative to carboxylation, so that the reduction of net assimilation rate due to photorespiration increases with  $T$ . Thus, the suppression of oxygenation by  $eC_a$  has a larger effect at higher temperatures. Hence, at the leaf scale, an interactive effect is expected between  $eC_a$  and  $T$ , as shown by Long (1991).

Most of the process-based growth and yield models start with photosynthesis; either treating it as the basic growth process underlying the carbon balance or using it as an independent predictor variable (Mäkelä et al. 2000). Many models of the response of vegetation to climate change incorporate  $eC_a \times T$  interaction effect on leaf photosynthesis. In the absence of any compensatory process, the interaction propagates through to larger scales. Using a forest canopy-scale model, McMurtrie and Wang (1993) showed there was a substantial rise in plant optimum growth temperature with increasing levels of  $C_a$ , because of increased assimilation rates but similar respiration costs. Using a global-scale model, Hickler et al. (2008) predicted the enhancement in net primary productivity (NPP) of forest ecosystems due to  $eC_a$  would increase with mean annual temperature (MAT). A positive interaction between  $eC_a$  and  $T$  is also predicted by models that take N cycling constraints into account (Medlyn et al. 2000, Pepper et al. 2005, Smith et al. 2014). In a recent model review, Medlyn et al. (2011) showed that this assumption is important in determining modelled climate impacts on productivity; models that do not incorporate an  $eC_a \times T$  interaction are

more likely to predict negative impacts on productivity than models that do incorporate the interaction.

Experimental results vary considerably in the type and magnitude of the response, and it is therefore not clear whether this model assumption is supported by the available observations. For example, a study by Teskey (1997) on 22-year old loblolly pine trees, showed that a 2°C increase in air temperature had far less effect on rates of carbon assimilation than an increase in  $C_a$  by 165  $\mu\text{mol mol}^{-1}$  or 330  $\mu\text{mol mol}^{-1}$ , and the  $eC_a$  and T effects were additive rather than interactive. Similarly, Norby and Luo (2004) did not find a significant interaction of  $eC_a$  and T on tree growth in two different species of maple. However, Lewis et al. (2013) did find a significant interaction between  $eC_a$  and T on plant stem biomass accumulation in two eucalyptus species.

Meta-analysis can help to discern trends in experimental data when results from individual experiments are contradictory. There have been two recent meta-analyses examining factorial  $eC_a \times T$  experiments, but neither directly tested for the positive interaction between the two factors predicted by models. Dieleman et al. (2012) reviewed a number of field-based factorial experiments with forests and grasslands and found that there were more antagonistic than synergistic effects in these experiments, but did not carry out a statistical test to establish the overall effect size. Wang et al. (2012) carried out a meta-analysis on a wide range of factorial  $eC_a \times T$  experiments, comparing the mean  $eC_a$  response across all low temperature treatments with the mean  $eC_a$  response across all high temperature treatments. They reported that in woody plants,  $eC_a$  stimulated biomass by a similar amount in ambient and elevated temperatures. However, this approach has low power because it does not take into account the pairing of control and manipulation treatments by experiment. Furthermore, this approach is flawed when the number of low-temperature  $eC_a$  responses does not equal the number of high-temperature  $eC_a$  responses (as in Wang et al. (2012)), because “low” and “high” temperatures are relative terms and therefore can only be applied to paired temperature treatments. No meta-analysis has so far directly examined the key model prediction that the  $eC_a$  response should be higher at locations with high MAT (Hickler et al. 2008).

In this paper, I used the meta-analysis approach to test specifically whether empirical data support the assumption of a positive interaction between  $eC_a$  and T that is embedded in many vegetation models. I carried out two meta-analyses. In the first meta-analysis, I examined factorial  $eC_a \times T$  experiments to test for an interaction-term between the  $eC_a$  and T treatments. In the second meta-analysis, I examined field-based experiments across the globe

to test the hypothesis that the  $eC_a$  effect on plant biomass increases with mean annual temperature.

## 2.2 Material and methods

### 2.2.1 $CO_2$ x temperature meta-analysis (Factorial experiments)

#### *Data collection*

Data were gathered by searching the ISI 'Web of Science' database for peer reviewed papers until December 2013 for elevated  $CO_2$  concentration x temperature factorial studies on woody species. These studies were located by searching the database using the search terms "elevated  $CO_2$  and temperature effect on plants", "high  $CO_2$  and high temperature effect on trees" and "elevated  $CO_2$  and warming effects on plant biomass". Data were taken from tables or digitized from figures, using the software "GetData Graph digitizer" (GetData Graph Digitizer 2008).

#### *Criteria for categorizing studies*

Database was constructed with plant biomass responses to the respective treatments with means, standard deviations and number of replicates. Factorial experiments had four treatments a) ambient  $CO_2$ , low temperature b) ambient  $CO_2$ , high temperature c) high  $CO_2$ , low temperature and d) high  $CO_2$ , high temperature. Studies were categorized with  $CO_2$  treatment range between 325-400  $\mu\text{mol mol}^{-1}$  for ambient levels, and 530-800  $\mu\text{mol mol}^{-1}$  for elevated levels. Factorial experiments had at least two temperature treatments in addition to two  $CO_2$  treatments. Most experiments used two temperature levels, where the 'high' temperature treatments were in the range 2°-5° C above 'low' or 'ambient' temperature treatments. There were four studies with more than two temperature treatments. For these studies I divided treatments into two independent pairs. Two of the studies had five temperature treatments; for these, I disregarded the lowest temperature treatment (4°C below ambient). For some studies, root biomass and shoot biomass were calculated from root to shoot ratio and total biomass. To weight these studies in the meta-analysis, I took standard deviations from the total biomass data. Some studies involved additional manipulations such as nutrient levels and different plant species. Results from these treatments within the same experiment were considered independent and were treated as independent responses in the

database. For experiments including watering treatments, only well-watered treatments were included, as low water availability may alter the  $eC_a \times$  temperature interaction. Several in-ground studies had to be omitted because there were no published estimates of above-ground or below-ground biomass increment. Studies used in this meta-analysis are listed in Table 1.

### Calculations

The  $eC_a \times$  temperature interaction term was calculated from factorial experiments as described by Lajeunesse (2011). If the mean is represented as  $\bar{X}$ ,  $C_e$  and  $C_a$  represent elevated and ambient  $CO_2$ , and  $T_e$  and  $T_a$  represent elevated and ambient temperature, then the interaction term in a factorial experiment can be written as the following response ratio:

$$r = \frac{\bar{X}_{C_e T_e} / \bar{X}_{C_e T_a}}{\bar{X}_{C_a T_e} / \bar{X}_{C_a T_a}} \quad (1)$$

To linearize this metric,  $r$  is log transformed to give

$$\ln(r) = \ln\left(\frac{\bar{X}_{C_e T_e}}{\bar{X}_{C_a T_e}}\right) - \ln\left(\frac{\bar{X}_{C_e T_a}}{\bar{X}_{C_a T_a}}\right) \quad (2)$$

That is, the log of the  $eC_a \times$  temperature interaction term is equal to the difference between the log of the  $CO_2$  response ratio at elevated temperature, and the log of the  $CO_2$  response ratio at ambient temperature. The response variable, % interaction effect or ‘effect size’, was calculated by taking antilog of  $\ln(r)$ , and then  $(r-1) \times 100$ . Hedges et al. (1999) showed that the variance ‘ $v$ ’ of a log response ratio at ambient temperature is given by

$$v = \frac{SD_{C_e T_a}^2}{n_{C_e T_a} \bar{X}_{C_e T_a}^2} + \frac{SD_{C_a T_a}^2}{n_{C_a T_a} \bar{X}_{C_a T_a}^2} \quad (3)$$

Using the additive property of variances, the variance of the log of the  $CO_2 \times$  temperature interaction term is equal to

$$v = \frac{SD_{C_e T_e}^2}{n_{C_e T_e} \bar{X}_{C_e T_e}^2} + \frac{SD_{C_e T_a}^2}{n_{C_e T_a} \bar{X}_{C_e T_a}^2} + \frac{SD_{C_a T_e}^2}{n_{C_a T_e} \bar{X}_{C_a T_e}^2} + \frac{SD_{C_a T_a}^2}{n_{C_a T_a} \bar{X}_{C_a T_a}^2} \quad (4)$$

To estimate an overall interaction term, weighted means were used, where greater weights were given to experiments whose estimates had greater precision (i.e., smaller variance). I used a random effects model because between-study variance was found to be statistically significant. The meta-analysis calculations were done using software R (R Development Core Team 2010) with package ‘metafor’ (Viechtbauer 2010).

### 2.2.2 Mean annual temperature meta-regression (Field experiments)

#### *Data collection*

The second type of study was field-based manipulative CO<sub>2</sub> enrichment experiments with woody species. These studies were also located by searching the ISI ‘Web of Science’ database for peer reviewed papers, with the terms used “elevated CO<sub>2</sub> effect on plants”, “high CO<sub>2</sub> effect on trees” and “elevated CO<sub>2</sub> effects on plant biomass”. Experiments had treatments with ambient CO<sub>2</sub> and elevated CO<sub>2</sub>. Only studies where trees were planted directly into the field were included (including open-top chamber, whole-tree chamber and free-air CO<sub>2</sub> enrichment experiments).

#### *Criteria for categorizing studies*

For studies where plants were grown from seed or seedlings, I used data on total biomass where available, or aboveground plant biomass where total plant biomass was not reported. In studies where plants were established prior to the experiment, the response variable was biomass increment or Net Primary Production or, in cases where neither variable was available, basal area increment. All Free-Air CO<sub>2</sub> Enrichment (FACE) studies had Net Primary Production data available except for the Sapporo, Japan FACE study. Studies were categorized with CO<sub>2</sub> treatment range between 325-400 µmol mol<sup>-1</sup> for ambient levels, and 530-800 µmol mol<sup>-1</sup> for elevated levels. Results from different plant species were considered to be independent and were treated as independent responses in the database. As in the first meta-analysis, I omitted drought treatments because low water availability may affect the eC<sub>a</sub> response. Studies used in this meta-analysis are listed in Table 2.

#### *Calculations*

For the second analysis, I carried out a meta-regression using the effect estimate of log response ratio of biomass as the outcome variable and mean annual temperature as the explanatory variable. To allow for the fact that the eC<sub>a</sub> concentration applied differed among experiments, which would interact with mean annual temperature, the meta-regression equation fitted was:

$$\ln(r) = \ln\left(\frac{eC_a}{aC_a}\right) \times (\alpha + \beta (MAT - 15)) \quad (5)$$

Where  $r$  is the observed response ratio,  $eC_a / aC_a$  is the percentage  $CO_2$  increase applied in the experiment, and  $\alpha$  and  $\beta$  are the fitted parameters. MAT was centred on  $15^\circ C$  to allow better estimation of the intercept  $\alpha$ .

Consistent mean annual temperatures for each experiment were estimated by extracting mean annual temperature for experimental site co-ordinates over the period 1991-2010 from a gridded monthly climatic data set (Harris et al., 2014). Individual studies were weighted by the inverse of variance of their respective effect size. Random-effects meta-regression was carried out using statistical programming software R (R Development Core Team 2010) with package 'metafor' (Viechtbauer 2010).

In the random-effects model, at least part of the heterogeneity may be due to the influence of moderators. For example, the response to  $eC_a$  concentration may depend on whether the studies are FACE or chamber-based; whether or not nutrients are added; and whether NPP or total plant biomass is used as the response variable. I examined the influence of these variables by fitting a mixed-effects model including FACE vs chamber, fertilized vs unfertilized NPP vs Biomass as moderators.



**Table 2-1:** List of Factorial eC<sub>a</sub> x temperature experiments used in meta-analysis, with study sites and location. Study codes were used to identify each study in meta-analysis forest plots.

Site	Location	Exp-code	Treatments	Species	TB	AGB	BGB	Source Paper
Athens	GA, USA	Athens		<i>Quercus rubra</i>	*			Bauweraerts et al, 2013
Corvallis	OR, USA	Corvallis		<i>Pseudotsuga menziesii</i>	*	*	*	Olszyk et al., 2003
Dahlem	Germany	Dahlem-1	-2 to 2° C	<i>Fagus sylvatica</i>	*			Overdieck et al., 2007
		Dahlem-2	0 to 4° C		*			"
Duke	NC, USA	Duke-1		<i>Pinus ponderosa</i>	*	*	*	Delucia et al., 1997
		Duke-2		<i>Pinus ponderosa</i>	*	*	*	Callaway et al., 1994
		Duke-3	High	<i>Robinia pseudoacacia</i>	*	*	*	Usselman et al., 2000
		Duke-4	Low		*	*	*	"
		Duke-5	High	<i>Pinus taeda</i>			*	King et al., 1996
		Duke-6	Low				*	"
		Duke-7	High	<i>Pinus ponderosa</i>			*	"
		Duke-8	Low				*	"
Flakaliden	Sweden	Flakaliden		<i>Picea abies</i>		*		Kostiainen et al., 2009
Harvard	MA,	Harvard		<i>Betula alleghaniensis</i>	*			Wayne et al., 1998
Horsholm	Denmark	Horsholm-1	-2 to 2.3° C	<i>Fagus sylvatica</i>	*	*	*	Bruhn and Saxe, 2000
		Horsholm-2	0 to 4.8° C		*	*	*	"
Mekrijarvi	Finland	Mekrijarvi-1		<i>Betula pendula</i>	*			Kuokkanen et al., 2001
		Mekrijarvi-2		<i>Betula pendula</i>	*			Kellomaki and Wang,
		Mekrijarvi-3		<i>Pinus sylvestris</i>		*		Sallas et al., 2003
		Mekrijarvi-4		<i>Salix myrsinifolia</i>		*		Veteli et al., 2002
		Mekrijarvi-5		<i>Betula pendula</i>	*	*	*	Lavola et al., 2013
Oak ridge	TN, USA	Oak ridge-1		<i>Acer rubrum</i>	*	*		Norby and Luo, 2004
		Oak ridge-2		<i>Acer saccharum</i>	*	*		"
		Oak ridge-3		<i>Acer</i>		*	*	Wan et al., 2004
Richmond	Australia	Richmond-1		<i>Eucalyptus saligna</i>	*	*	*	Ghannoum et al., 2010
		Richmond-2		<i>Eucalyptus sideroxylon</i>	*	*	*	"
		Richmond-3		<i>Eucalyptus saligna</i>	*	*	*	Lewis et al., 2013
		Richmond-4		<i>Eucalyptus sideroxylon</i>	*	*	*	"
		Richmond-5		<i>Eucalyptus globulus</i>	*	*	*	Duan et al., 2013
Saerheim	Norway	Saerheim		<i>Betula pubescens</i>	*	*	*	Mortenson, 1995
Shanghai	China	Shanghai		<i>Abies faxoniana</i>	*	*	*	Hou et al., 2010
Taichung	Taiwan	Taichung		<i>Shima superba</i>	*			Sheu and Lin, 1999
Tsukuba	Japan	Tsukuba		<i>Quercus myrsinaefolia</i>	*	*	*	Usami et al., 2001
Urbana	IL, USA	Urbana		<i>Pinus ponderosa</i>	*	*	*	Maherali and Delucia,
St. Paul	MN,	St. Paul_1	21°C - 24°C	<i>Picea mariana</i>	*			Tjoelker et al., 1998
		St. Paul_2	27°C - 30°C	<i>Picea mariana</i>	*			"
		St. Paul_3	21°C - 24°C	<i>Pinus banksina</i>	*			"
		St. Paul_4	27°C - 30°C	<i>Pinus banksina</i>	*			"
		St. Paul_5	21°C - 24°C	<i>Larix laricina</i>	*			"
		St. Paul_6	27°C - 30°C	<i>Larix laricina</i>	*			"
		St. Paul_7	21°C - 24°C	<i>Betula papyrifera</i>	*			"
		St. Paul_8	27°C - 30°C	<i>Betula papyrifera</i>	*			"

\* denotes whether the study reported TB = Total Biomass, AGB = Above Ground Biomass and/or BGB = Below Ground Biomass.

**Table 2-2:** List of eC<sub>a</sub> experiments with trees freely rooted in the ground used in meta-analysis.

Obs.	Site name	Location	Type of Experiment	Species	Nutrients	Other treatment	Parameter	Mean Annual Temperature °C	Reference paper
1	Bangor	UK	FACE	<i>Alnus glutinosa</i>			Above	10.2	Smith et al., 2013
2			FACE	<i>Betula pendula</i>			Above		
3			FACE	<i>Fagus sylvatica</i>			Above		
4	Birmendorf	Switzerland	OTC	<i>Fagus sylvatica</i>	High	Acidic soil	Total	9.5	Spinnler et al. 2002
5			OTC	<i>Fagus sylvatica</i>	Low	Acidic soil	Total		
6			OTC	<i>Fagus sylvatica</i>	High	Calcareous soil	Total		
7			OTC	<i>Fagus sylvatica</i>	Low	Calcareous soil	Total		
8			OTC	<i>Picea abies</i>	High	Acidic soil	Total		
9			OTC	<i>Picea abies</i>	Low	Acidic soil	Total		
10			OTC	<i>Picea abies</i>	High	Calcareous soil	Total		
11			OTC	<i>Picea abies</i>	Low	Calcareous soil	Total		
12	Bungendore	Australia	OTC	<i>Eucalyptus pauciflora</i>			Total	12.7	Roden et al. 1999
13			OTC*	<i>Eucalyptus pauciflora</i>		grown with grasses	Total		Loveys et al. 2010
14			OTC	<i>Eucalyptus pauciflora</i>		shading of chambers	Total		Barker et al. 2005
15	Darwin	Australia	CTC	<i>Mangifera indica</i>			Total	27.2	Goodfellow et al. 1997
16	Davos	Switzerland	FACE	<i>Larix decidua</i>			Shoot	1.8	Dawes et al., 2011
17			FACE	<i>Pinus mugo</i>			Shoot	1.8	
18	Duke	NC, USA	FACE	<i>Pinus taeda</i>			Total NPP	15.3	McCarthy et al, 2010
19			OTC	<i>Pinus taeda</i>			Total		Tissue et al. 1997
20	Flakaliden	Sweden	WTC	<i>Picea abies</i>			Above	2	Sigurdsson et al., 2013
21			WTC	<i>Picea abies</i>	High		Above		
22			WTC	<i>Picea abies</i>	Low		Above		
23	Glencorse	UK	OTC*	<i>Betula pendula</i>			Total	8.3	Rey and Jarvis. 1998
24	Glendevon	UK	OTC	<i>Alnus glutinosa</i>	High		Total	8.1	Temperton et al., 2003
25			OTC	<i>Alnus glutinosa</i>	Low		Total		
26			OTC	<i>Betula pendula</i>	High		Total		ECOCRAFT, 1999
27			OTC	<i>Betula pendula</i>	Low		Total		
28			OTC	<i>Pinus sylvestris</i>	High		Total		
29			OTC	<i>Pinus sylvestris</i>	Low		Total		
30			OTC	<i>Picea sitchensis</i>	High		Total		
31			OTC	<i>Picea sitchensis</i>	Low		Total		
32	Gunnesholt	Iceland	WTC	<i>Populus trichocarpa</i>	High		Total	5.2	Sigurdsson et al. 2001
33			WTC	<i>Populus trichocarpa</i>	Low		Total		

Obs.	Site name	Location	Type of Experiment	Species	Nutrients	Other treatment	Parameter	Mean Annual Temperature °C	Reference paper
34	Headley	UK	OTC	<i>Quercus petraea</i>			Total	10	
35			OTC	<i>Quercus rubra</i>			Total		
36			OTC	<i>Fraxinus excelsior</i>			Total		Broadmeadow et al., 2000
37			OTC	<i>Quercus petraea</i>			Total		
38			OTC	<i>Pinus sylvestris</i>			Total		
39	Hyderabad	India	OTC	<i>Gmelina arborea</i>			Total	27	Reddy et al. 2010
40	Merritt	FA, USA	OTC	<i>Quercus myrtifolia</i> /			Above	22.4	Day et al., 2013
41	Mekrijarvi	Finland	CTC	<i>Pinus sylvestris</i>			Biomass	2.5	Paltola et al, 2002
42	Oak ridge	TN, USA	OTC	<i>Acer rubrum</i>			Total	14.6	Norby et al., 2000
43			OTC	<i>Acer saccharum</i>			Total		
44			FACE	<i>Liquidambar styraciflua</i>			Total NPP		Norby et al., 2010
45			OTC	<i>Quercus alba</i>		350 to 500 $\mu\text{mol mol}^{-1} \text{CO}_2$	Total		Norby et al., 1995
46			OTC	<i>Quercus alba</i>		350 to 650 $\mu\text{mol mol}^{-1} \text{CO}_2$	Total		Norby et al., 1995
47			OTC	<i>Liriodendron tulipifera</i>		Ambient to Ambient + 150	Total		Norby et al., 1992
48			OTC	<i>Liriodendron tulipifera</i>		Ambient to Ambient + 300	Total		
49	Parque Natural	Panama	OTC	Tree communities			Biomass	26.3	Lovelock et al., 1998
50	Phoenix	AR, USA	OTC*	<i>Pinus eldarica</i>		554 $\mu\text{mol mol}^{-1} \text{CO}_2$	Total	21.9	Idso & Kimball 1994
51			OTC*	<i>Pinus eldarica</i>		680 $\mu\text{mol mol}^{-1} \text{CO}_2$	Total		
52			OTC*	<i>Pinus eldarica</i>		812 $\mu\text{mol mol}^{-1} \text{CO}_2$	Total		
53			OTC	<i>Citrus aurantium</i>			Total		Kimball et al. 2007
54	Placerville	NV, USA	OTC	<i>Pinus ponderosa</i>	High		Total	14.1	Johnson et al. 1997
55			OTC	<i>Pinus ponderosa</i>	Low		Total		
56			OTC	<i>Pinus ponderosa</i>	High		Total		
57			OTC	<i>Pinus ponderosa</i>	Low		Total		
58			OTC	<i>Pinus ponderosa</i>	Medium		Total		
59	Rhineland	WI, USA	FACE	<i>Populus tremuloides</i>			Total NPP	4.3	King et al., 2005
60			FACE	<i>Populus tremuloides</i> /			Total NPP		
				<i>Betula papyrifera</i>					
61	Richmond	Australia	WTC	<i>Eucalyptus saligna</i>			Total	17	Barton et al, 2011
62	Saporo	Japan	FACE	<i>Larix gmelinii</i>			Total	7.6	Watanabe et al., 2013
63			FACE	<i>Larix gmelinii</i>			Total		
64	Suonenjoki	Finland	OTC	<i>Betula pendula</i>		O3-tolerant (Clone 4)	Total	3.8	Riikonen et al.2004
65			OTC	<i>Betula pendula</i>		O3-sensitive clones (Clone 80)	Total		
66	Technical	Germany	Mini-ecosystem	<i>Fagus sylvatica</i>	High		Biomass	13.8	Forstreuter 1995
67	UIA	Belgium	OTC	<i>Pinus sylvestris</i>	Low		Total	10.8	Janssens et al. 2005

Obs.	Site name	Location	Type of Experiment	Species	Nutrients	Other treatment	Parameter	Mean Annual Temperature °C	Reference paper
68			OTC	<i>Poplar Beaupre</i>			Biomass	10.8	Ceulemans et al., 1996
69			OTC	<i>Poplar Robusta</i>			Biomass	10.8	
70	UMBS	MI, USA	OTC	<i>Populus tremuloides</i>	High		Total	5.9	Zak et al. 2000
71			OTC	<i>Populus tremuloides</i>	Low		Total		
72			OTC	<i>Populus tremuloides</i>	High		Total		Mikan et al. 2000
73			OTC	<i>Populus tremuloides</i>	Low		Total		
74			OTC	<i>Alnus glutinosa</i>			Total		Vogel et al. 1997
75			OTC	<i>Populus euramericana</i>	High		Total		Pregitzer et al. 1995
76			OTC	<i>Populus euramericana</i>	Low		Total		
77			OTC	<i>Populus grandidentata</i>			Total		Zak et al. 1993
78	UPS	France	Mini-ecosystem	<i>Fagus sylvatica</i>			Biomass	15	Badeck et al., 1997
79	Vielsalm	Belgium	OTC	<i>Picea abies</i>			Biomass	7.5	Laitat et al., 1994
80	Viterbo	Italy	FACE	<i>Populus euramericana</i>			Total NPP	16	Calfapietra et al., 2003
81			FACE	<i>Populus alba</i>			Total NPP		
82			FACE	<i>Populus nigra</i>			Total NPP		

Abbreviations: FACE=free air carbon dioxide enrichment, OTC=open top chamber, CTC=closed top chambers, WTC= whole tree chambers, NPP= net primary productivity. \* indicates studies which had single tree in treatment chambers.

### 2.2.3 *Baseline model predictions*

I used model simulations to predict the magnitude of effect sizes as a baseline against which to compare the meta-analysis results. For the first meta-analysis, I used two photosynthesis models to estimate the expected effect sizes of an increase in  $C_a$ , an increase in temperature, and the interaction between the two effects. The first model was the standard biochemical leaf photosynthesis model of Farquhar & von Caemmerer (1982). Calculations were made for both the Rubisco limited reaction ( $A_c$ ) and the RuBP-regeneration limited reaction ( $A_j$ ). I took temperature dependences for the Michaelis-Menten coefficients of Rubisco ( $K_c$  and  $K_o$ ) and the  $CO_2$  compensation point in the absence of mitochondrial respiration ( $\Gamma^*$ ) from Bernacchi et al. (2001). The activation energies of maximum Rubisco activity,  $V_{cmax}$ , and potential electron transport,  $J_{max}$ , were taken to be 58.52 and 37.87 KJ mol<sup>-1</sup> respectively, following Medlyn et al. (2002), while leaf day respiration ( $R_d=0.015 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was assumed to have a Q10 of 2.

The second model was the optimised net canopy photosynthesis model of Haxeltine and Prentice (1996), which is used in the LPJ family of Dynamic Global Vegetation Models (Sitch et al. 2003). This model is based on the Collatz et al. (1991) simplification of the Farquhar model and assumes that leaf N content varies to maximise net canopy photosynthesis, resulting in an “acclimation” of  $V_{cmax}$  to growth conditions including temperature and  $eC_a$ . This model was parameterised with values from Haxeltine & Prentice (1996).

Using both models, I calculated photosynthesis at two levels of  $CO_2$  ( $370 \mu\text{mol mol}^{-1}$  and  $690 \mu\text{mol mol}^{-1}$ ) and two temperatures (16 to  $20.5^\circ\text{C}$ ); where these levels of  $CO_2$  and temperature represent the mean values of  $CO_2$  and temperature used in the factorial experiments. From these outputs I calculated the expected size of the  $eC_a$  and T effects and the  $eC_a \times T$  interaction.

To obtain baseline predictions of the NPP enhancement at varying mean annual temperatures across the globe for the second meta-analysis, I ran global simulations using two Dynamic Global Vegetation Models (DGVMs), the JULES model (Best et al. 2011, Clark et al. 2011), and the O-CN model (Zaehle et al. 2010, Zaehle et al. 2011) following as far as possible the simulation protocol of Hickler et al. (2008). I also took baseline predictions from simulations with the LPJ DGVM by Hickler et al. (2008) (their Figure A1). The JULES simulations were driven with the WATCH-forcing data based on the ERA interim climatology ([http://www.eu-watch.org/data\\_availability](http://www.eu-watch.org/data_availability)), at 0.5 degree spatial resolution and 3 hourly time step. The JULES model was run with fixed land use, calculated for the JULES

plant functional types based on the MODIS in IGBP land cover map. The O-CN simulations at 1 degree spatial resolution and a half-hourly time step were based on simulations from 1860 until 1995 driven with the daily CRU-NCEP climate data set, the observed atmospheric CO<sub>2</sub> record, reconstructed land-use change, and an estimate of N deposition, as described in Le Quéré et al. (2013). The simulation were then continued for the period 1996-2002 (with inter annual climate variation but static land-cover and N deposition from 1996) for either holding C<sub>a</sub> constant at 1996 value or with a step increase to 550 μmol mol<sup>-1</sup>.

For the analyses of this chapter, non-forest pixels were excluded for all three models. Hickler et al. (2008) ran the LPJ-model with potential natural vegetation and included only grid cells that carry natural forests other than savanna. Grid cells with very low NPP (< 100 g m<sup>-2</sup> yr<sup>-1</sup>) and Woody LAI of <0.5 for boreal forests and 2.5 for the remaining forests were also excluded for those cells which did not predict forest biomes. Following the same protocol, for the O-CN model, I excluded pixels which had predicted NPP < 100 g m<sup>-2</sup> yr<sup>-1</sup>; pixels with less than 25% forest cover in total; and pixels with LAI < 2.5 where latitude < 60°N or LAI < 1 where latitude > 60°N. Similarly, for the JULES model, pixels were excluded where NPP < 100 g m<sup>-2</sup> yr<sup>-1</sup> or where forest cover < 25% ([http://daac.ornl.gov/NPP/guides/NPP\\_BOREAL.html#HDataDescrAccess](http://daac.ornl.gov/NPP/guides/NPP_BOREAL.html#HDataDescrAccess)). Subsequently, savannahs were also removed by using the dominant vegetation type map from Ramankutty and Foley (1999). As there are default LAI fields used in the JULES model which are specific for broad-leaf or needle-leaf, no LAI filtering was done.

## 2.3 Results

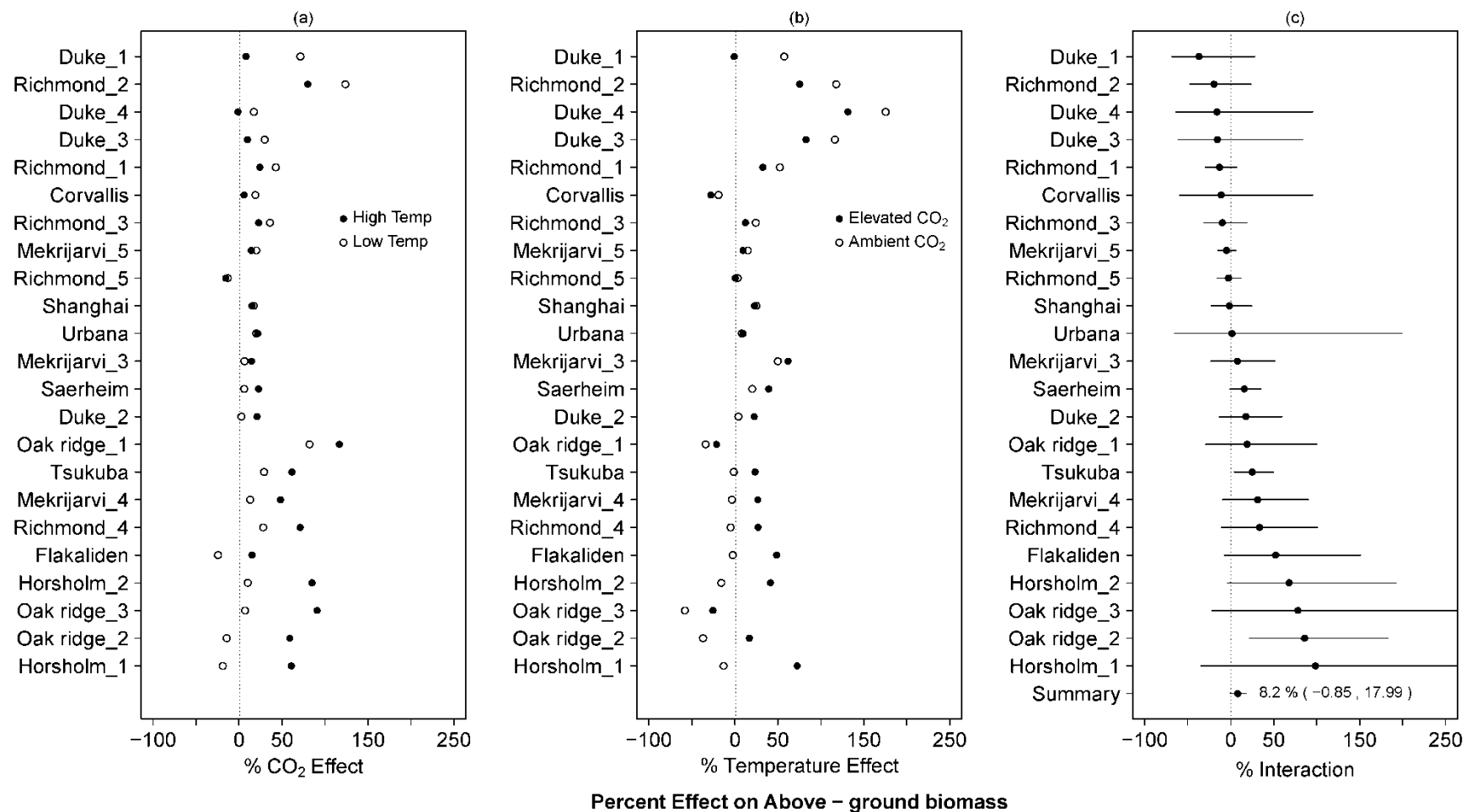
### 2.3.1 Factorial experiments

Out of 42 experiments, I could obtain above-ground biomass for 23 experiments, either directly from data reported or by calculating it from root: shoot ratio and total biomass. Of these 23 experiments, 16 observations were total above-ground biomass and 7 were stem biomass. I also obtained 22 observations for plant below-ground biomass and 32 for total biomass responses (Table 2.1). For plant above-ground biomass there were significant positive mean effects of both eC<sub>a</sub> (mean effect size +21.4%) and temperature (mean effect size +18.1%) (Figure 2.1a, b, Table 2.3). The effect size of eC<sub>a</sub> was consistent with the value predicted by the leaf-level A<sub>j</sub> and canopy photosynthesis models, but was significantly less than that predicted by the leaf A<sub>c</sub> model (Table 2.3). Most studies showed a positive effect of

eC<sub>a</sub> (Figure 2.1a) whereas there was more variation among studies in the temperature effect (Figure 2.1b). Rising temperature may have positive or negative effects depending on whether plants are above or below their temperature optimum. For the interaction term, the mean effect size was +8.2% (95% CI = -0.85, 18.0). This effect was not significantly different from zero ( $p = 0.08$ ), but it was also not significantly different from the effect sizes predicted by the leaf and canopy models, which were in the range 3.5 – 8.3% (Table 3).

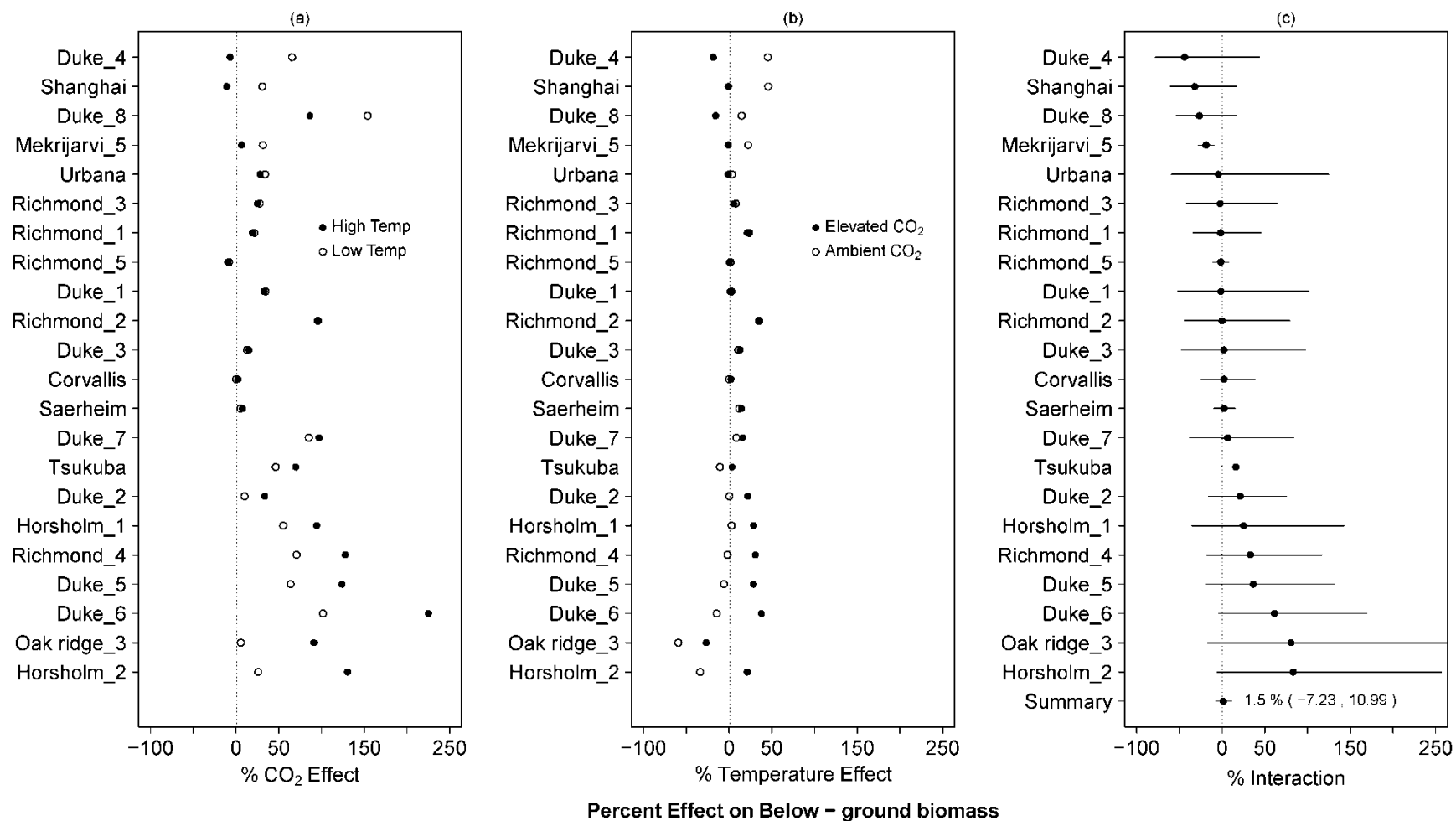
Similar results were found for below-ground and total biomass plant responses. For below-ground biomass, a slightly larger mean eC<sub>a</sub> effect (+35.2%) was observed whereas the temperature effect was +6.6%. The mean eC<sub>a</sub> x temperature interaction was positive, but not significantly different from zero (+1.5%, Figure 2.2c). For total biomass, eC<sub>a</sub> had a positive effect (+22.3%), as did increased temperature (+7.7%) while the mean eC<sub>a</sub> x temperature interaction was +0.5%, with a 95% CI of (-8.02, 9.8). Large confidence intervals were observed for individual studies in plant total biomass responses (Figure 2.3c) due to within study and between study variations (between-group heterogeneity  $Q$  ( $df = 31$ ) = 84.8,  $p$ -value < 0.0001).

Although the interaction term was not significantly different from zero for any response variables, the 95% confidence intervals also included the interaction sizes predicted by the leaf-scale and canopy-scale models (Table 2.3). Using the Farquhar & von Caemmerer (1982) photosynthesis model, I predicted that under RuBP-regeneration limitation, the percentage increases of photosynthesis in response to eC<sub>a</sub>, temperature and their interaction would be +16%, +16.5% and +3.5%, respectively, indicating that the size of the eC<sub>a</sub> x T interaction is relatively small. The 95% confidence intervals found in the meta-analysis for the effect sizes include these effect sizes. However, when Rubisco activity is assumed to limit photosynthesis, the predicted eC<sub>a</sub> effect (+44.6%) is above the observed CIs for above-ground and total biomass (Table 2.3). The eC<sub>a</sub> effect and eC<sub>a</sub> x T interaction effect predicted by the canopy-scale model are comparable to the RuBP-regeneration limited response, and also fall within the observed confidence intervals, but the model predicts a reduction (-7.3%) in photosynthesis with an increase in temperature, which disagrees with observations (Table 2.3).

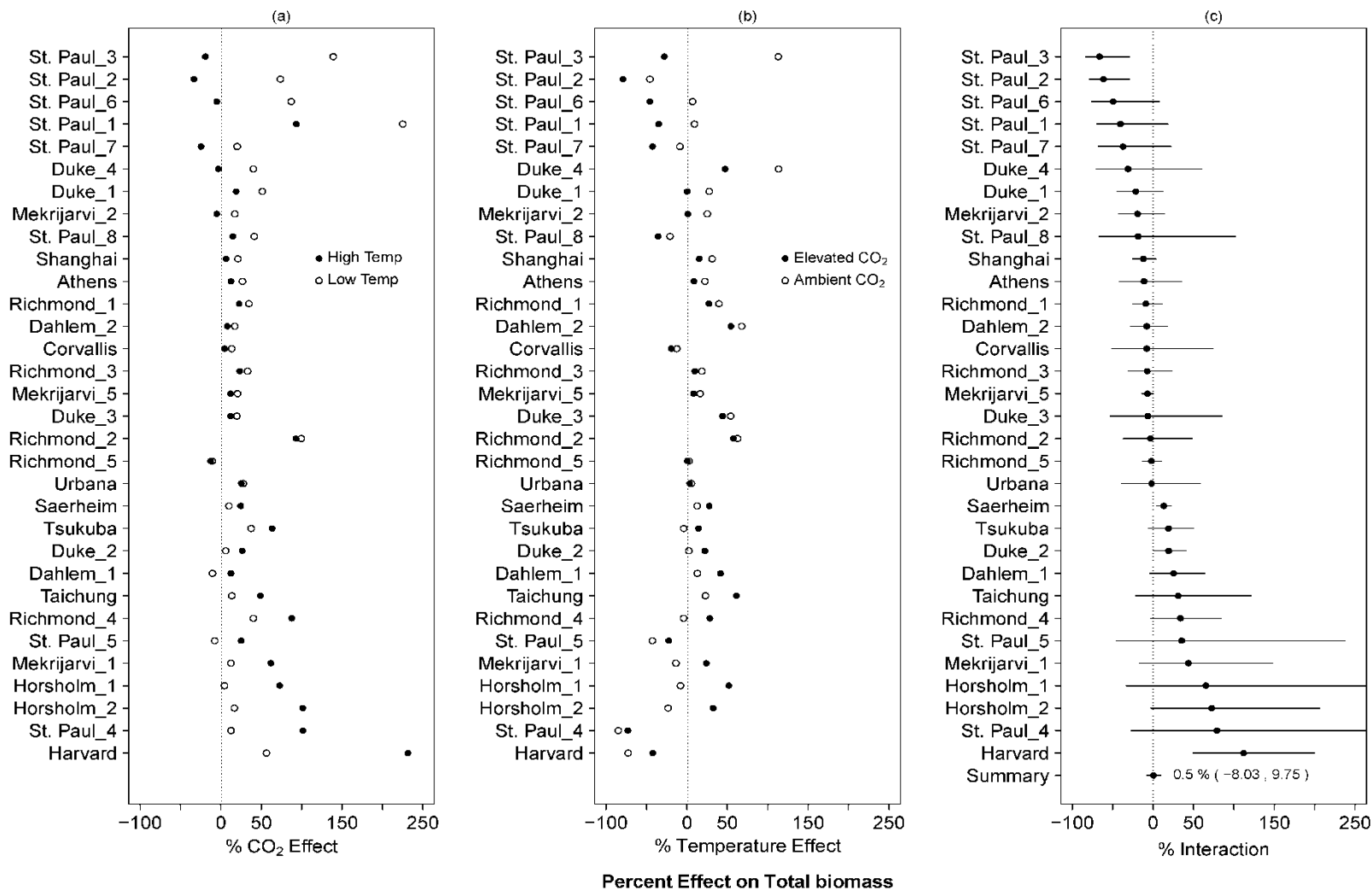


**Figure 2-1:** Forest plots of standardized effect sizes for (a) the elevated CO<sub>2</sub> (eC<sub>a</sub>) effect at low and high temperature; (b) the temperature effect at ambient and elevated CO<sub>2</sub>; and (c) the eC<sub>a</sub> x temperature interaction term for above ground plant biomass in eC<sub>a</sub> x temperature factorial experiments. Each point represents the mean effect size of an individual study, apart from the last point in (c) which shows the mean (summary) effect size of all studies. Lines in (c) indicate 95% confidence intervals. The dashed vertical line shows zero effect. Studies are ordered by the eC<sub>a</sub> x temperature interaction size.





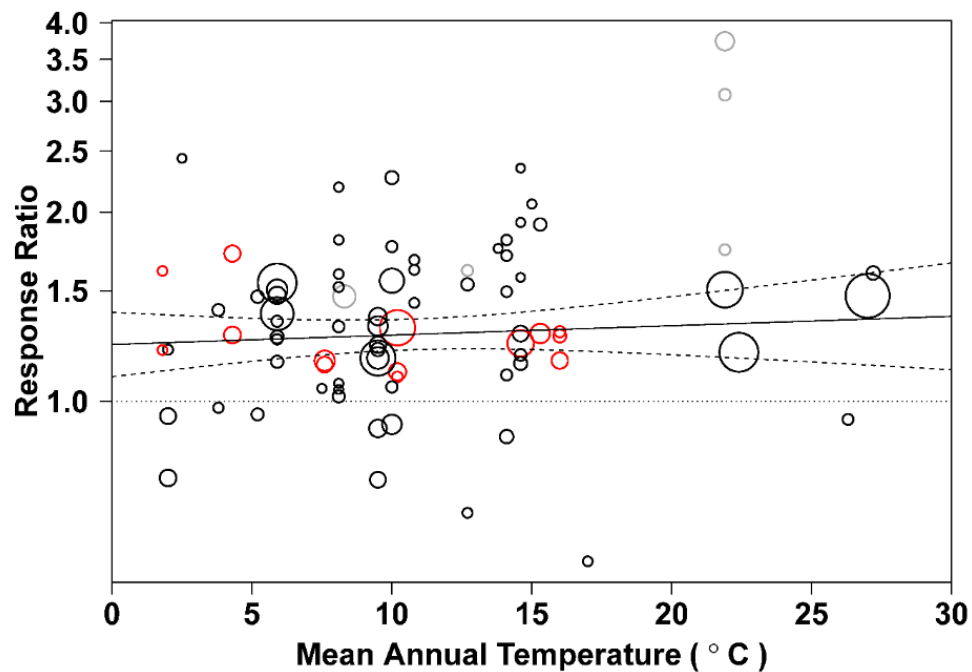
**Figure 2-2:** As for Figure 2.1, but for below-ground plant biomass.



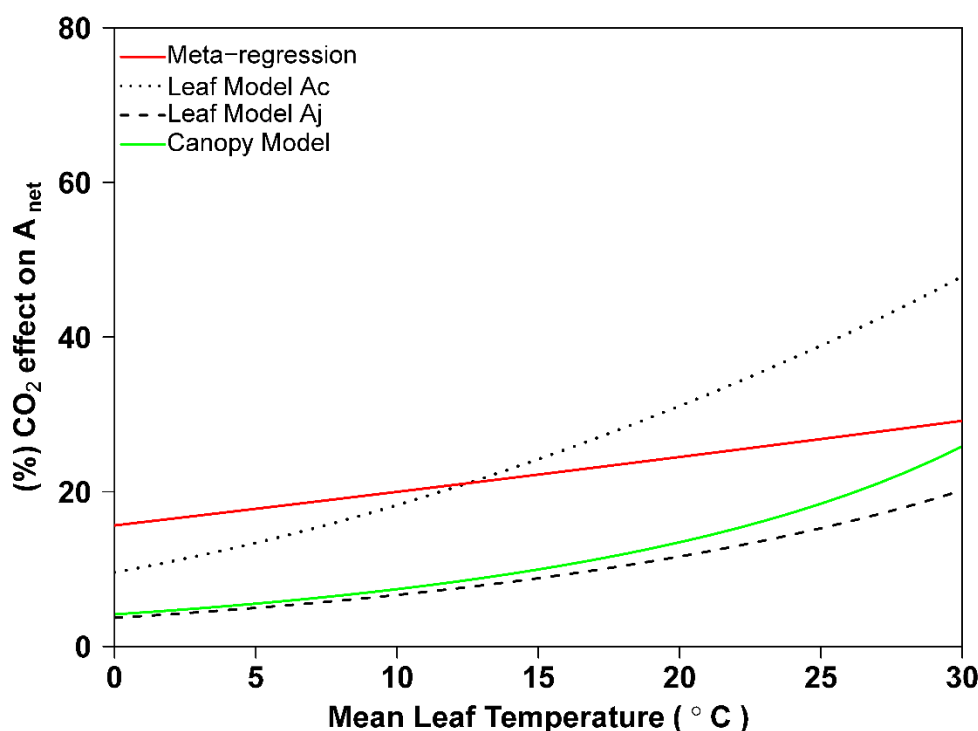
**Figure 2-3:** As for Figure 2.1, but for total plant biomass.

### 2.3.2 Field experiments

For my second analysis, data were obtained from 82 studies around the globe in which trees were planted directly into the ground and exposed to  $aC_a$  or  $eC_a$  concentrations (Table 2.2). The response ratio for these studies was calculated from measures of total biomass, above-ground biomass, net primary production, or basal area increment, depending on the information available for each experiment. I carried out a meta-regression of the log response ratio in these studies against mean annual temperature of the site, using a random effects model, in which larger weight (indicated by larger circles in Figure 2.4) is given to studies with lower variance.



**Figure 2-4:** Meta-regression of the response ratio of total biomass to  $eC_a$  in field-based experiments with woody species, against mean annual temperature. The area of each circle is inversely proportional to the variance of the log response ratio estimate and indicates the weighting assigned to each study. The dotted line shows zero or no effect, the solid black line represents the linear meta-regression line (slope=0.0034,  $p>0.05$ ) for studies in which trees were grown in groups and dashed black lines show predicted effects with corresponding confidence interval bounds. Grey circles represent single tree studies (refer to table 2.2). Red circles denote data from FACE (Free-Air  $CO_2$  Enrichment) experiments. Note that y-axis is log transformed.



**Figure 2-5:** Meta-regression relationship with  $\text{CO}_2$  increment =  $190 \mu\text{mol mol}^{-1}$ , compared to modelled percentage response of net photosynthesis to the same increase in  $C_a$  as a function of mean leaf temperature. Solid red line: meta-regression. Dotted line: modelled response of Rubisco-limiting leaf net photosynthetic rate ( $A_c$ ). Dashed line: modelled response of RuBP-regeneration-limited leaf net photosynthetic rate ( $A_j$ ). Both  $A_c$  and  $A_j$  were calculated according to the Farquhar & von Caemmerer (1982) model. Solid green line: modelled response of net daily canopy photosynthesis according to the Haxeltine & Prentice (1996) model.

When all studies were included, there was a statistically significant relationship between the response ratio and mean annual temperature. However, it appeared that this relationship was being driven by a single experiment on *Pinus eldarica* trees (Idso and Kimball 1994). The response ratios found in this experiment were clear outliers and may have been caused by the fact that, in contrast to most other experiments, trees were grown singly in treatment chambers, with no competition from other trees. I therefore excluded all studies (see Table 2.2) that had single trees in treatment chambers (five studies; grey points in Figure 2.4). When these studies were excluded, the slope of the meta-regression against mean annual temperature remained positive (0.0087, CI= -0.007, 0.0249), but was no longer significantly different from zero (Figure 2.4). Slope coefficients for the regression including  $\text{CO}_2$  increment are given in Table 2.4.

I tested whether the relationship was affected by experimental factors by including additional factors in the meta-regression. I used dummy variables to test whether the relationship differed between FACE and chamber studies, fertilized vs non-fertilised studies or whether the relationship differed for NPP vs total plant biomass. None of the three factors had a significant effect on the slope.

**Table 2-3:** Comparison between meta-analytic and modelled estimates of percentage effects of  $eC_a$ , temperature and their interaction in factorial experiments. Meta-analysis values are mean effect sizes with 95% CIs. The Farquhar & von Caemmerer (1982) model was used to estimate effects on net leaf photosynthesis when Rubisco activity is limiting ( $A_c$ ) or when RuBP regeneration is limiting ( $A_j$ ). The model of Haxeltine & Prentice (1996) was used to estimate effects on canopy net photosynthesis.

	% $eC_a$ effect	% Temperature effect	% $eC_a \times$ temperature
<b>Meta-analysis:</b>			
Above-ground biomass	21.4% (11.01, 32.8)	18.1% (9.3, 27.7)	8.2% (-0.85, 17.99)
Below-ground biomass	35.2% (18.8, 53.9)	6.6% (1.02, 12.5)	1.5% (-7.2, 10.9)
Total biomass	22.3% (13.9-31.4)	7.7% (-1.4-17.7)	0.5% (-8.02-9.8)
<b>Models:</b>			
Leaf $A_c$	44.6%	15.9%	8.3%
Leaf $A_j$	16.0%	16.5%	3.5%
Canopy	19.5%	-7.3%	4.7%

**Table 2-4:** Results of meta-regression with log  $CO_2$  response ratio of plant biomass as dependent variable, and mean annual temperature (MAT, °C) and  $CO_2$  increment ( $CO_2$  inc,  $\mu mol\ mol^{-1}$ ) as independent variables. Table 2.shows Coefficient (estimate), standard error (SE), 95% confidence interval (CI) and p-value.

	Coefficient	SE	CI	p-value
$\ln(CO_2\ inc + 360/360)$	0.4735	0.0615	0.3529 0.5941	<.0001
MAT - 15	0.0087	0.0082	-0.0074 0.0249	0.289

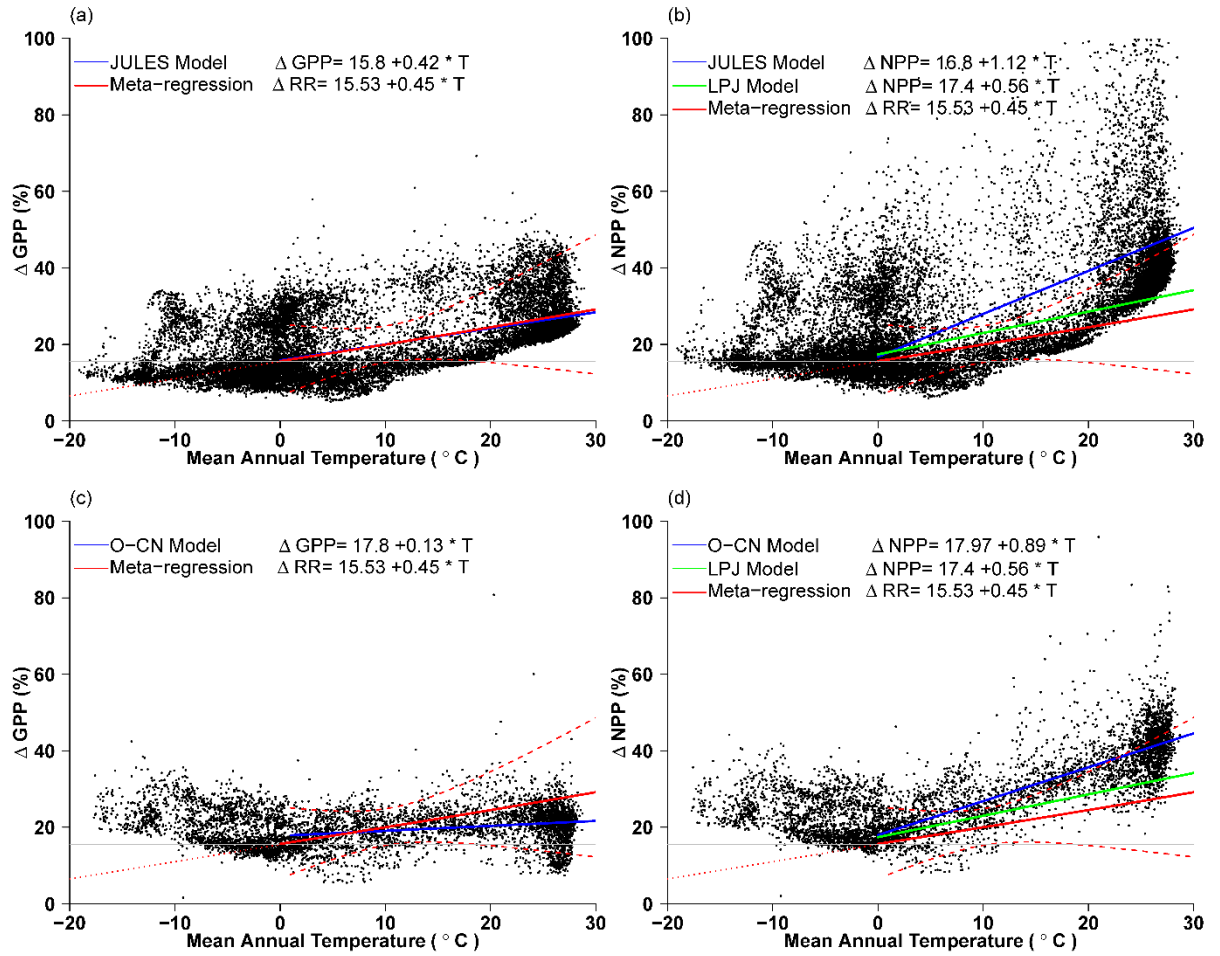
### 2.3.3 Comparison with baseline model predictions

To investigate how the response obtained from meta-analysis compares to model predictions, I compared the meta-regression relationship with outcomes from the two photosynthesis models (Figure 2.5) and the three DGVMs (Figure 2.6). The comparison to the leaf/canopy level models in Figure 2.5 is indicative only, since it compares the modelled  $CO_2$  response of photosynthesis at a given instantaneous temperature, against measured biomass responses integrating the seasonal course of temperatures, at the reference mean annual temperature. The response obtained with the Haxeltine & Prentice (1996) model is very close

to the response obtained for RuBP-regeneration-limited photosynthesis (see also Table 2.3). Both are less steep than the response of Rubisco-limited photosynthesis, which is more sensitive to temperature due to the high temperature sensitivity of the  $K_m$  of Rubisco. All model-based response curves are steeper than the meta-regression relationship.

In Figure 2.6, I compare the meta-regression relationship with GPP enhancements predicted by the JULES and O-CN models, and with NPP enhancements predicted by these models and LPJ-DGVM, which relies on the Haxeltine & Prentice (1996) model to simulate photosynthesis. The GPP enhancement is lower at all mean annual temperatures in the O-CN model than in the JULES model (Figure 2.6a, c), possibly due to combination of a fraction of photosynthesis that is light limited (i.e.  $A_j$ -limited photosynthesis) as well as gradual acclimation of foliar N due to limited N supply under  $eC_a$  in the O-CN model. Both models show an increasing  $C_a$  response with mean annual temperatures above 0°C. I fitted linear regressions for the model output for pixels with MAT > 0°C (Figure 6). The slope of the response in JULES is very similar to the slope of the meta-regression, but the slope of the response is less steep in O-CN. Interestingly, both models appear to show that the predicted  $eC_a$  response of GPP increases as MAT decreases below 0°C. When plotted against growing season temperature rather than MAT, however, the relationship is monotonically positive (not shown), suggesting that locations with extremely low MAT may also have relatively high growing season temperature, possibly due to a continentality effect. There have been no experiments in locations with MAT below the 0°C threshold to date, so there are no data against which to compare this response.

The NPP response of both models is larger, and more strongly related to temperature, than the GPP response (Figure 2.6b, d). The relationship is similar in the O-CN and LPJ models, but steeper in the JULES model. Of the three models, the relationship predicted by the LPJ model is closest to the meta-regression. As with the GPP responses, the NPP responses in JULES and O-CN increase with decreasing MAT below 0°C. This does not occur in the LPJ model, Figure 2.A1), where the CO<sub>2</sub> enhancement continues to decrease below 0°C. However, at MAT close to 0°C, all of the models predict NPP enhancements considerably above those predicted by the meta-regression. The implications of these comparisons between model predictions and meta-analysis are considered in the discussion below.



**Figure 2-6:** Comparison of Meta-regression relationship with DGVM predictions of  $\text{CO}_2$  enhancement of GPP (a, c) and NPP (b, d). Data points are output from the JULES model (a, b) and O-CN model (c, d). Solid red line: meta-regression relationship. Dashed red line: confidence intervals for meta-regression. Dotted red line: fitted-linear regression line. Solid green line: relationship fitted to output from LPJ model (Hickler et al. 2008). Grey line represents zero effect with respect to meta-regression (red) line.

## 2.4 Discussion

In this study I focused on the question of whether  $C_a$  responses of plant growth are larger at higher temperatures. I designed two methods to address this question. Firstly, I looked at factorial  $eC_a \times$  temperature experiments and analysed whether there is an interaction; and secondly, I analysed whether there is a trend in  $C_a$  response across experiments with different mean annual temperatures. In both analyses, variability among and within experiments was sufficiently large that confidence intervals included both zero and the modelled effect size. The experimental data available to date therefore do not allow to

distinguish between the competing hypotheses of a positive interaction of  $eC_a$  and temperature on growth, and no interaction.

#### 2.4.1 Factorial experiments

Applying meta-analysis to the factorial experiments, I found an overall positive, but non-significant  $eC_a$  x temperature interaction for plant above-ground, below-ground and total biomass (Table 2.3). However, the confidence intervals also included the predicted interaction size for light-limited and canopy-scale photosynthesis, meaning that we cannot statistically reject the possibility that an interaction exists. For the size of the temperature increase typically applied in factorial experiments, the predicted interaction term is very small compared to  $C_a$  or temperature effect (+3.5 to +8.3%, Table 3) and compared to variability among replicates. Very few individual experiments have sufficient power to detect an effect of this size. Combining experiments in meta-analysis often increases power, enabling small effects to be detected, but high variability among experiments may counteract this increase in power.

Variability amongst the factorial  $eC_a$  x T experiments in this meta-analysis was high, likely caused by a range of experimental design factors. In some experiments, temperature levels were held constant, while in others, temperatures varied with the ambient temperature. Plant material varied widely, from boreal to subtropical species, with some species grown at below-optimal temperatures and others grown at or above their optimal temperatures. In some studies, additional nutrients were provided to reduce nutrient stress, while others did not add nutrients. Experiments also varied in the length of time that plants were exposed to  $eC_a$  (60 days to 4 years), the age at which treatment started (0-8 years old) and whether plants were freely rooted or grown in pots. With a limited number of experimental datasets, and such a wide range of experimental conditions, it was not possible to conclusively identify the factors responsible for variation among experiments.

Previous meta-analyses did not find evidence for a significant interaction between  $eC_a$  and temperature (Dieleman et al. 2012, Wang et al. 2012), but these analyses did not test whether the interaction term was significantly different from that predicted by models. By determining confidence intervals for the interaction effect size, we show that it is not possible to reject the hypothesis of a positive  $eC_a$  x T interaction as predicted by models based on these experiments. The chief reason for the small, observation-based interaction term is that the temperature increments applied in the factorial experiments were relatively small (typically +2 to +5°C). To increase the chance of detecting an interactive effect, it may be appropriate to



consider factorial experiments with larger temperature increments. For a 10°C increase in temperature from 20°C to 30°C, for example, the predicted interaction effect size rises to 10% for  $A_j$  and 20% for  $A_c$ . However, such experiments would need to be conducted with caution, as there is a high potential for experimental artifacts with larger changes in temperatures.

#### 2.4.2 *Field experiments*

In the second meta-analysis I compared  $eC_a$  responses from experiments with trees around the globe, giving a much larger range in growth temperature. I attempted to include all published experiments, but some high-profile experiments had to be omitted from this analysis because there was no estimate of  $eC_a$  effect on biomass increment or NPP that was comparable with other studies. The Swiss webFACE experiment (Bader et al. 2013) on a mature deciduous forest is one such experiment; however, the uncertainty bounds on stem growth for that experiment were sufficiently large (Fatichi and Leuzinger 2013) that inclusion of that experiment, had it been possible, would not have affected the outcome of the regression.

The second meta-analysis was also inconclusive. I did not find a statistically significant relationship between the  $eC_a$  responses of plant biomass production and mean annual temperature. However, there was high variability among experiments and the 95% CI for the meta-regression included the relationships predicted by three DGVMs, meaning it was not possible to reject the interaction effect sizes embedded in the models.

Comparison of the meta-regression with model outputs needs to be interpreted with caution as the model outputs do not exactly coincide with the experiments. The experiments were conducted on a range of different experimental material but principally on young, rapidly expanding trees competing for water and nutrients from neighbouring trees, whereas the DGVMs simulated the effects of a step change in  $C_a$  on established forests. In young, rapidly growing plants, leaf area feedbacks amplify the response of photosynthesis, and these feedbacks may be more pronounced at high temperatures. This effect will not be captured in the DGVMs. On the other hand, in the DGVMs, the slope of the NPP response vs MAT is much steeper than the GPP response vs MAT (Figure 2.6) because respiration is estimated from plant biomass, and in established forests the  $C_a$  effect on plant biomass lags behind the effect on GPP. This effect is amplified at high temperatures. Following a step change in  $C_a$ , therefore, the slope of the NPP response vs MAT relationship predicted by DGVMs is steep, but the slope diminishes over time. The latter effect will not be present in experiments on young trees.

Despite this incompatibility between the experiments and model outputs, we can nonetheless draw some useful observations from the comparison.

Firstly, the comparison helps to understand causes for the differences among the models. The LPJ model predicts lower CO<sub>2</sub> responses than the JULES model, as has been observed previously (Sitch et al. 2008). At a MAT of 20°C, the JULES model predicts an average 39.2% increase in NPP whereas the LPJ-model predicts only 28.6% increase in NPP (Hickler et al. 2008). This difference likely arises because of the use of the Haxeltine & Prentice (1996) photosynthesis model in LPJ, in which  $V_{\text{cmax}}$  acclimates to  $eC_a$ , reducing the  $eC_a$  effect compared to JULES which uses the Farquhar photosynthesis model without acclimation (Figure 2.5).

Secondly, the comparison highlights the need for experiments in a wider range of growing temperatures. Although the  $eC_a$  experiments included in the second meta-analysis cover a much wider range of temperature than the factorial  $eC_a \times T$  experiments, they are nonetheless largely restricted to zones with MAT between 5°C and 15°C (Figure 2.4). Very few data are available for the largest forested regions – the boreal zone and the tropics – underscoring the need for further experiments investigating CO<sub>2</sub> responses in these regions.

New experiments are needed not only to investigate whether the interaction between  $eC_a$  and  $T$  on plant biomass production exists, but also to explore the potential mechanisms that might cause the interaction not to occur. Such mechanisms might include acclimation of photosynthesis and/or respiration to growth temperature, or feedbacks via water or nutrient availability. If, with further experiments, we are able to statistically reject the  $eC_a \times T$  interaction currently predicted by models, it will be important to modify the models accordingly. To do so, we will need to identify the most important mechanisms causing the leaf-level interaction to be over-ridden at whole-plant scale. Comparison of experimental data against model predictions, as done here, will be key for identifying such mechanisms.

## *Conclusion*

In conclusion, neither of the meta-analyses that I performed allowed to distinguish between the two competing hypotheses of a positive  $eC_a \times$  temperature interaction, and no interaction. Until further data become available, it would be useful for modelling studies to indicate how this uncertainty affects projected responses to climate change by evaluating the consequences of both hypotheses.

## References

- Ainsworth, E. A. and S. P. Long. 2005. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist* 165:351-371.
- Badeck F.-W., D. E., Epron D., Le Dantec V., Liozon R., Mousseau M., Pontailier J.-Y. and Saugier B. 1997. Sweet chestnut and beech saplings under elevated CO<sub>2</sub>. Pages 15-25. *in* K. K. a. S. S. G.M.J. Mohren, editor. *Impacts of Global Change on Tree Physiology and Forest Ecosystems*. Kluwer Academic Publishers, Dordrecht.
- Bader, M. K. F., S. Leuzinger, S. G. Keel, R. T. W. Siegwolf, F. Hagedorn, P. Schleppi, and C. Körner. 2013. Central European hardwood trees in a high-CO<sub>2</sub> future: synthesis of an 8-year forest canopy CO<sub>2</sub> enrichment project. *Journal of Ecology* 101:1509-1519.
- Barker, D. H., B. R. Loveys, J. J. G. Egerton, H. Gorton, W. E. Williams, and M. C. Ball. 2005. CO<sub>2</sub> enrichment predisposes foliage of a eucalypt to freezing injury and reduces spring growth. *Plant Cell and Environment* 28:1506-1515.
- Barnett, T. P., J. C. Adam, and D. P. Lettenmaier. 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* 438:303-309.
- Barton, C. V. M., R. A. Duursma, B. E. Medlyn, D. S. Ellsworth, D. Eamus, D. T. Tissue, M. A. Adams, J. Conroy, K. Y. Crous, M. Liberloo, M. Low, S. Linder, and R. E. McMurtrie. 2012. Effects of elevated atmospheric [CO<sub>2</sub>] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. *Global Change Biology* 18:585-595.
- Bauweraerts, I., T. M. Wertin, M. Ameye, M. A. McGuire, R. O. Teskey, and K. Steppe. 2013. The effect of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology* 19:517-528.
- Bernacchi, C. J., E. L. Singaas, C. Pimentel, A. R. Portis, and S. P. Long. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell and Environment* 24:253-259.
- Best, M. J., M. Pryor, D. B. Clark, G. G. Rooney, R. L. H. Essery, C. B. Ménard, J. M. Edwards, M. A. Hendry, A. Porson, N. Gedney, L. M. Mercado, S. Sitch, E. Blyth, O. Boucher, P. M. Cox, C. S. B. Grimmond, and R. J. Harding. 2011. The Joint UK Land Environment Simulator (JULES), model description – Part 1: Energy and water fluxes. *Geosci. Model Dev.* 4:677-699.
- Broadmeadow, M. S. J. and S. B. Jackson. 2000. Growth responses of *Quercus petraea*, *Fraxinus excelsior* and *Pinus sylvestris* to elevated carbon dioxide, ozone and water supply. *New Phytologist* 146:437-451.
- Bruhn, D., J. W. Leverenz, and H. Saxe. 2000. Effects of tree size and temperature on relative growth rate and its components of *Fagus sylvatica* seedlings exposed to two partial pressures of atmospheric [CO<sub>2</sub>]. *New Phytologist* 146:415-425.
- Calfapietra, C., B. Gielen, A. N. J. Galema, M. Lukac, P. De Angelis, M. C. Moscatelli, R. Ceulemans, and G. Scarascia-Mugnozza. 2003. Free-air CO<sub>2</sub> enrichment (FACE) enhances biomass production in a short-rotation poplar plantation. *Tree Physiology* 23:805-814.
- Callaway, R. M., E. H. Delucia, E. M. Thomas, and W. H. Schlesinger. 1994. Compensatory Responses of CO<sub>2</sub> Exchange and Biomass Allocation and Their Effects on the Relative Growth-Rate of Ponderosa Pine in Different CO<sub>2</sub> and Temperature Regimes. *Oecologia* 98:159-166.
- Canadell, J. G. and M. R. Raupach. 2008. Managing forests for climate change mitigation. *Science* 320:1456-1457.
- Ceulemans, R., B. Y. Shao, X. N. Jiang, and J. Kalina. 1996. First- and second-year aboveground growth and productivity of two *Populus* hybrids grown at ambient and elevated CO<sub>2</sub>. *Tree Physiology* 16:61-68.
- Clark, D. B., L. M. Mercado, S. Sitch, C. D. Jones, N. Gedney, M. J. Best, M. Pryor, G. G. Rooney, R. L. H. Essery, E. Blyth, O. Boucher, R. J. Harding, C. Huntingford, and P. M. Cox. 2011. The Joint UK Land Environment Simulator (JULES), model description – Part 2: Carbon fluxes and vegetation dynamics. *Geosci. Model Dev.* 4:701-722.

- Collatz, G. J., J. T. Ball, C. Grivet, and J. A. Berry. 1991. Physiological and Environmental-Regulation of Stomatal Conductance, Photosynthesis and Transpiration - a Model That Includes a Laminar Boundary-Layer. *Agricultural and Forest Meteorology* 54:107-136.
- Dawes, M. A., S. Hattenschwiler, P. Bebi, F. Hagedorn, I. T. Handa, C. Korner, and C. Rixen. 2011. Species-specific tree growth responses to 9 years of CO<sub>2</sub> enrichment at the alpine treeline. *Journal of Ecology* 99:383-394.
- Day, F. P., R. E. Schroeder, D. B. Stover, A. L. P. Brown, J. R. Butnor, J. Dilustro, B. A. Hungate, P. Dijkstra, B. D. Duval, T. J. Seiler, B. G. Drake, and C. R. Hinkle. 2013. The effects of 11 yr of CO<sub>2</sub> enrichment on roots in a Florida scrub-oak ecosystem. *New Phytologist* 200:778-787.
- Delucia, E. H., R. M. Callaway, E. M. Thomas, and W. H. Schlesinger. 1997. Mechanisms of phosphorus acquisition for ponderosa pine seedlings under high CO<sub>2</sub> and temperature. *Annals of Botany* 79:111-120.
- Dieleman, W. I. J., S. Vicca, F. A. Dijkstra, F. Hagedorn, M. J. Hovenden, K. S. Larsen, J. A. Morgan, A. Volder, C. Beier, J. S. Dukes, J. King, S. Leuzinger, S. Linder, Y. Q. Luo, R. Oren, P. de Angelis, D. Tingey, M. R. Hoosbeek, and I. A. Janssens. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO<sub>2</sub> and temperature. *Global Change Biology* 18:2681-2693.
- Duan, H., J. S. Amthor, R. A. Duursma, A. P. O'Grady, B. Choat, and D. T. Tissue. 2013. Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO<sub>2</sub>] and elevated temperature. *Tree Physiology* 33:779-792.
- ECOCRAFT. 1999. Predicted impacts of rising carbon dioxide and temperature on forests in Europe at stand scale. Institute of Ecology and Resource Management, University of Edinburgh, Edinburgh, UK.
- Farquhar, G. D. and S. Caemmerer. 1982. Modelling of Photosynthetic Response to Environmental Conditions. Pages 549-587 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. *Physiological Plant Ecology II*. Springer Berlin Heidelberg.
- Farquhar, G. D., S. Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta* 149:78-90.
- Fatichi, S. and S. Leuzinger. 2013. Reconciling observations with modeling: The fate of water and carbon allocation in a mature deciduous forest exposed to elevated CO<sub>2</sub>. *Agricultural and Forest Meteorology* 174:144-157.
- Fatichi, S., S. Leuzinger, and C. Korner. 2014. Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. *New Phytologist* 201:1086-1095.
- Forstreuter, M. 1995. Bestandesstruktur und Netto-Photosynthese von jungen Buchen (*Fagus sylvatica* L.) unter erhoehter CO<sub>2</sub>-Konzentration VERHANDLUNGEN- GESELLSCHAFT FUR OKOLOGIE 24:283-292.
- GetData Graph Digitizer. 2008. <http://getdata-graph-digitizer.com/>.
- Ghannoum, O., N. G. Phillips, J. P. Conroy, R. A. Smith, R. D. Attard, R. Woodfield, B. A. Logan, J. D. Lewis, and D. T. Tissue. 2010. Exposure to preindustrial, current and future atmospheric CO<sub>2</sub> and temperature differentially affects growth and photosynthesis in Eucalyptus. *Global Change Biology* 16:303-319.
- Goodfellow, J., D. Eamus, and G. Duff. 1997. Diurnal and seasonal changes in the impact of CO<sub>2</sub> enrichment on assimilation, stomatal conductance and growth in a long-term study of *Mangifera indica* in the wet-dry tropics of Australia. *Tree Physiology* 17:291-299.
- Harris, I., P. D. Jones, T. J. Osborn, and D. H. Lister. 2014. Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology* 34:623-642.
- Haxeltine, A. and I. C. Prentice. 1996. A general model for the light-use efficiency of primary production. *Functional Ecology* 10:551-561.
- Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80:1150-1156.
- Hickler, T., B. Smith, I. C. Prentice, K. Mjofors, P. Miller, A. Arneth, and M. T. Sykes. 2008. CO<sub>2</sub> fertilization in temperate FACE experiments not representative of boreal and tropical forests. *Global Change Biology* 14:1531-1542.

- Hou, Y., Z. K. Luo, G. D. Jenerette, Y. Z. Qiao, and K. Y. Wang. 2010. Effects of Elevated CO<sub>2</sub> and Temperature on Growth and Morphology of Fir (*Abies Faxoniana* Rehd. Et Wils.) and Native Herbs in a Treeline Ecotone: An Experimental Approach. *Polish Journal of Ecology* 58:311-322.
- Hyvonen, R., G. I. Agren, S. Linder, T. Persson, M. F. Cotrufo, A. Ekblad, M. Freeman, A. Grelle, I. A. Janssens, P. G. Jarvis, S. Kellomaki, A. Lindroth, D. Loustau, T. Lundmark, R. J. Norby, R. Oren, K. Pilegaard, M. G. Ryan, B. D. Sigurdsson, M. Stromgren, M. van Oijen, and G. Wallin. 2007. The likely impact of elevated [CO<sub>2</sub>], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist* 173:463-480.
- Idso, S. B. and B. A. Kimball. 1994. Effects of Atmospheric CO<sub>2</sub> Enrichment on Biomass Accumulation and Distribution in Eldarica Pine Trees. *Journal of Experimental Botany* 45:1669-1672.
- IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.
- Janssens, I. A., B. Medlyn, B. Gielen, I. Laureysens, M. E. Jach, D. Van Hove, and R. Ceulemans. 2005. Carbon budget of *Pinus sylvestris* saplings after four years of exposure to elevated atmospheric carbon dioxide concentration. *Tree Physiology* 25:325-337.
- Johnson, D. W., J. T. Ball, and R. F. Walker. 1997. Effects of CO<sub>2</sub> and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. *Plant and Soil* 190:29-40.
- Kellomaki, S. and K. Y. Wang. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. *Annals of Botany* 87:669-682.
- Kimball, B. A., S. B. Idso, S. Johnson, and M. C. Rillig. 2007. Seventeen years of carbon dioxide enrichment of sour orange trees: final results. *Global Change Biology* 13:2171-2183.
- King, J. S., M. E. Kubiske, K. S. Pregitzer, G. R. Hendrey, E. P. McDonald, C. P. Giardina, V. S. Quinn, and D. F. Karnosky. 2005. Tropospheric O<sub>3</sub> compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO<sub>2</sub>. *New Phytologist* 168:623-635.
- King, J. S., R. B. Thomas, and B. R. Strain. 1996. Growth and carbon accumulation in root systems of *Pinus taeda* and *Pinus ponderosa* seedlings as affected by varying CO<sub>2</sub>, temperature and nitrogen. *Tree Physiology* 16:635-642.
- Kirschbaum, M. U. F. 2011. Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO<sub>2</sub> Enrichment Studies. *Plant Physiology* 155:117-124.
- Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202-2205.
- Kostiainen, K., S. Kaakinen, P. Saranpaa, B. D. Sigurdsson, S. O. Lundqvist, S. Linder, and E. Vapaavuori. 2009. Stem wood properties of mature Norway spruce after 3 years of continuous exposure to elevated [CO<sub>2</sub>] and temperature. *Global Change Biology* 15:368-379.
- Kuokkanen, K., R. Julkunen-Tiitto, M. Keinanen, P. Niemela, and J. Tahvanainen. 2001. The effect of elevated CO<sub>2</sub> and temperature on the secondary chemistry of *Betula pendula* seedlings. *Trees-Structure and Function* 15:378-384.
- Laitat, E., Loosveldt, P., Boussard, H. and Hirvijarvi, E. 1994. Study on major morphological, physiological and biochemical processes likely to be affected under combined effects of increasing atmospheric CO<sub>2</sub> concentrations and elevated temperature in partial ecosystem enclosures. Pages 37-52. in R. C. F. Veroustraete, I. Impens and J. Van Rensbergen, editor. *Vegetation, Modelling and Climate Change Effects*. SPB Academic Publishing, The Hague, The Netherlands.
- Lajeunesse, M. J. 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology* 92:2049-2055.

- Lavola, A., L. Nybakken, M. Rousi, J. Pusenius, M. Petrelius, S. Kellomaki, and R. Julkunen-Tiitto. 2013. Combination treatment of elevated UVB radiation, CO<sub>2</sub> and temperature has little effect on silver birch (*Betula pendula*) growth and phytochemistry. *Physiologia Plantarum* 149:499-514.
- Le Quéré, C., G. P. Peters, R. J. Andres, R. M. Andrew, T. Boden, P. Ciais, P. Friedlingstein, R. A. Houghton, G. Marland, R. Moriarty, S. Sitch, P. Tans, A. Arneeth, A. Arvanitis, D. C. E. Bakker, L. Bopp, J. G. Canadell, L. P. Chini, S. C. Doney, A. Harper, I. Harris, J. I. House, A. K. Jain, S. D. Jones, E. Kato, R. F. Keeling, K. Klein Goldewijk, A. Körtzinger, C. Koven, N. Lefèvre, A. Omar, T. Ono, G. H. Park, B. Pfeil, B. Poulter, M. R. Raupach, P. Regnier, C. Rödenbeck, S. Saito, J. Schwinger, J. Segschneider, B. D. Stocker, B. Tilbrook, S. van Heuven, N. Viovy, R. Wanninkhof, A. Wiltshire, S. Zaehle, and C. Yue. 2013. Global carbon budget 2013. *Earth Syst. Sci. Data Discuss.* 6:689-760.
- Lewis, J. D., R. A. Smith, O. Ghannoum, B. A. Logan, N. G. Phillips, and D. T. Tissue. 2013. Industrial-age changes in atmospheric [CO<sub>2</sub>] and temperature differentially alter responses of faster- and slower-growing Eucalyptus seedlings to short-term drought. *Tree Physiology* 33:475-488.
- Linderholm, H. W. 2006. Growing season changes in the last century. *Agricultural and Forest Meteorology* 137:1-14.
- Long, S. P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO<sub>2</sub> concentrations: Has its importance been underestimated? *Plant, Cell & Environment* 14:729-739.
- Lovelock, C. E., K. Winter, R. Mersits, and M. Popp. 1998. Responses of communities of tropical tree species to elevated CO<sub>2</sub> in a forest clearing. *Oecologia* 116:207-218.
- Loveys, B. R., J. J. G. Egerton, D. Bruhn, and M. C. Ball. 2010. Disturbance is required for CO<sub>2</sub>-dependent promotion of woody plant growth in grasslands. *Functional Plant Biology* 37:555-565.
- Maherali, H. and E. H. DeLucia. 2000. Interactive effects of elevated CO<sub>2</sub> and temperature on water transport in ponderosa pine. *American Journal of Botany* 87:243-249.
- Mäkelä, A., J. Landsberg, A. R. Ek, T. E. Burk, M. Ter-Mikaelian, G. I. Agren, C. D. Oliver, and P. Puttonen. 2000. Process-based models for forest ecosystem management: current state of the art and challenges for practical implementation. *Tree Physiology* 20:289-298.
- McCarthy, H. R., R. Oren, K. H. Johnsen, A. Gallet-Budynnek, S. G. Pritchard, C. W. Cook, S. L. LaDeau, R. B. Jackson, and A. C. Finzi. 2010. Re-assessment of plant carbon dynamics at the Duke free-air CO<sub>2</sub> enrichment site: interactions of atmospheric [CO<sub>2</sub>] with nitrogen and water availability over stand development. *New Phytologist* 185:514-528.
- Mcmurtrie, R. E. and Y. P. Wang. 1993. Mathematical-Models of the Photosynthetic Response of Tree Stands to Rising CO<sub>2</sub> Concentrations and Temperatures. *Plant Cell and Environment* 16:1-13.
- Medlyn, B. E., R. A. Duursma, and M. J. B. Zeppel. 2011. Forest productivity under climate change: a checklist for evaluating model studies. *Wiley Interdisciplinary Reviews-Climate Change* 2:332-355.
- Medlyn, B. E., D. Loustau, and S. Delzon. 2002. Temperature response of parameters of a biochemically based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster Ait.*). *Plant Cell and Environment* 25:1155-1165.
- Medlyn, B. E., R. E. McMurtrie, R. C. Dewar, and M. P. Jeffreys. 2000. Soil processes dominate the long-term response of forest net primary productivity to increased temperature and atmospheric CO<sub>2</sub> concentration. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 30:873-888.
- Mikan, C. J., D. R. Zak, M. E. Kubiske, and K. S. Pregitzer. 2000. Combined effects of atmospheric CO<sub>2</sub> and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. *Oecologia* 124:432-445.
- Mortensen, L. M. 1995. Effect of Carbon-Dioxide Concentration on Biomass Production and Partitioning in *Betula-Pubescens Ehrh* Seedlings at Different Ozone and Temperature Regimes. *Environmental Pollution* 87:337-343.

- Norby, R. J., C. A. Gunderson, S. D. Wullschleger, E. G. Oneill, and M. K. McCracken. 1992. Productivity and Compensatory Responses of Yellow-Poplar Trees in Elevated CO<sub>2</sub>. *Nature* 357:322-324.
- Norby, R. J., J. S. Hartz-Rubin, and M. J. Verbrugge. 2003. Phenological responses in maple to experimental atmospheric warming and CO<sub>2</sub> enrichment. *Global Change Biology* 9:1792-1801.
- Norby, R. J., T. M. Long, J. S. Hartz-Rubin, and E. G. O'Neill. 2000. Nitrogen resorption in senescing tree leaves in a warmer, CO<sub>2</sub>-enriched atmosphere. *Plant and Soil* 224:15-29.
- Norby, R. J. and Y. Q. Luo. 2004. Evaluating ecosystem responses to rising atmospheric CO<sub>2</sub> and global warming in a multi-factor world. *New Phytologist* 162:281-293.
- Norby, R. J., J. M. Warren, C. M. Iversen, B. E. Medlyn, and R. E. McMurtrie. 2010. CO<sub>2</sub> enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America* 107:19368-19373.
- Norby, R. J., S. D. Wullschleger, C. A. Gunderson, and C. T. Nietch. 1995. Increased Growth Efficiency of *Quercus-Alba* Trees in a CO<sub>2</sub>-Enriched Atmosphere. *New Phytologist* 131:91-97.
- Olszyk, D. M., M. G. Johnson, D. T. Tingey, P. T. Rygielwicz, C. Wise, E. VanEss, A. Benson, M. J. Storm, and R. King. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO<sub>2</sub> and temperature for 4 years. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 33:269-278.
- Overdieck, D., D. Ziche, and K. Bottcher-Jungclaus. 2007. Temperature responses of growth and wood anatomy in European beech saplings grown in different carbon dioxide concentrations. *Tree Physiology* 27:261-268.
- Peltola, H., A. Kilpelainen, and S. Kellomaki. 2002. Diameter growth of Scots pine (*Pinus sylvestris*) trees grown at elevated temperature and carbon dioxide concentration under boreal conditions. *Tree Physiology* 22:963-972.
- Pepper, D. A., S. J. Del Grosso, R. E. McMurtrie, and W. J. Parton. 2005. Simulated carbon sink response of shortgrass steppe, tallgrass prairie and forest ecosystems to rising [CO<sub>2</sub>], temperature and nitrogen input. *Global Biogeochemical Cycles* 19.
- Pregitzer, K. S., D. R. Zak, P. S. Curtis, M. E. Kubiske, J. A. Teeri, and C. S. Vogel. 1995. Atmospheric CO<sub>2</sub>, Soil-Nitrogen and Turnover of Fine Roots. *New Phytologist* 129:579-585.
- R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramankutty, N. and J. A. Foley. 1999. Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles* 13:997-1027.
- Reddy, A. R., G. K. Rasineni, and A. S. Raghavendra. 2010. The impact of global elevated CO<sub>2</sub> concentration on photosynthesis and plant productivity. *Current Science* 99:46-57.
- Rey, A. and P. G. Jarvis. 1997. Growth Response of Young Birch Trees (*Betula pendula* Roth.) After Four and a Half Years of CO<sub>2</sub> Exposure. *Annals of Botany* 80:809-816.
- Reyer, C., P. Lasch-Born, F. Suckow, M. Gutsch, A. Murawski, and T. Pilz. 2014. Projections of regional changes in forest net primary productivity for different tree species in Europe driven by climate change and carbon dioxide. *Annals of Forest Science* 71:211-225.
- Riikonen, J., M. M. Lindsberg, T. Holopainen, E. Oksanen, J. Lappi, P. Peltonen, and E. Vapaavuori. 2004. Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiology* 24:1227-1237.
- Roden, J. S., J. J. G. Egerton, and M. C. Ball. 1999. Effect of elevated [CO<sub>2</sub>] on photosynthesis and growth of snow gum (*Eucalyptus pauciflora*) seedlings during winter and spring. *Australian Journal of Plant Physiology* 26:37-46.
- Sallas, L., E. M. Luomala, J. Utriainen, P. Kainulainen, and J. K. Holopainen. 2003. Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. *Tree Physiology* 23:97-108.
- Sheu, B. H. and C. K. Lin. 1999. Photosynthetic response of seedlings of the sub-tropical tree *Schima superba* with exposure to elevated carbon dioxide and temperature. *Environmental and Experimental Botany* 41:57-65.

- Sigurdsson, B. D., J. L. Medhurst, G. Wallin, O. Eggertsson, and S. Linder. 2013. Growth of mature boreal Norway spruce was not affected by elevated [CO<sub>2</sub>] and/or air temperature unless nutrient availability was improved. *Tree Physiology* 33:1192-1205.
- Sigurdsson, B. D., H. Thorgeirsson, and S. Linder. 2001. Growth and dry-matter partitioning of young *Populus trichocarpa* in response to carbon dioxide concentration and mineral nutrient availability. *Tree Physiology* 21:941-950.
- Sitch, S., C. Huntingford, N. Gedney, P. E. Levy, M. Lomas, S. L. Piao, R. Betts, P. Ciais, P. Cox, P. Friedlingstein, C. D. Jones, I. C. Prentice, and F. I. Woodward. 2008. Evaluation of the terrestrial carbon cycle, future plant geography and climate-carbon cycle feedbacks using five Dynamic Global Vegetation Models (DGVMs). *Global Change Biology* 14:2015-2039.
- Sitch, S., B. Smith, I. C. Prentice, A. Arneth, A. Bondeau, W. Cramer, J. O. Kaplan, S. Levis, W. Lucht, M. T. Sykes, K. Thonicke, and S. Venevsky. 2003. Evaluation of ecosystem dynamics, plant geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. *Global Change Biology* 9:161-185.
- Smith, A. R., M. Lukac, R. Hood, J. R. Healey, F. Miglietta, and D. L. Godbold. 2013. Elevated CO<sub>2</sub> enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist* 198:156-168.
- Smith, B., D. Wårlind, A. Arneth, T. Hickler, P. Leadley, J. Siltberg, and S. Zaehle. 2014. Implications of incorporating N cycling and N limitations on primary production in an individual-based dynamic vegetation model. *Biogeosciences* 11:2027-2054.
- Spinnler, D., P. Egh, and C. Korner. 2002. Four-year growth dynamics of beech-spruce model ecosystems under CO<sub>2</sub> enrichment on two different forest soils. *Trees-Structure and Function* 16:423-436.
- Taylor, G., M. J. Tallis, C. P. Giardina, K. E. Percy, F. Miglietta, P. S. Gupta, B. Gioli, C. Calfapietra, B. Gielen, M. E. Kubiske, G. E. Scarascia-Mugnozza, K. Kets, S. P. Long, and D. F. Karnosky. 2008. Future atmospheric CO<sub>2</sub> leads to delayed autumnal senescence. *Global Change Biology* 14:264-275.
- Temperton, V. M., S. J. Grayston, G. Jackson, C. V. M. Barton, P. Millard, and P. G. Jarvis. 2003. Effects of elevated carbon dioxide concentration on growth and nitrogen fixation in *Alnus glutinosa* in a long-term field experiment. *Tree Physiology* 23:1051-1059.
- Teskey, R. O. 1997. Combined effects of elevated CO<sub>2</sub> and air temperature on carbon assimilation of *Pinus taeda* trees. *Plant Cell and Environment* 20:373-380.
- Tissue, D. T., R. B. Thomas, and B. R. Strain. 1997. Atmospheric CO<sub>2</sub> enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. *Plant Cell and Environment* 20:1123-1134.
- Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO<sub>2</sub> and temperature. *Tree Physiol* 18:715-726.
- Usami, T., J. Lee, and T. Oikawa. 2001. Interactive effects of increased temperature and CO<sub>2</sub> on the growth of *Quercus myrsinaefolia* saplings. *Plant Cell and Environment* 24:1007-1019.
- Usselman, S. M., R. G. Qualls, and R. B. Thomas. 2000. Effects of increased atmospheric CO<sub>2</sub>, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant and Soil* 222:191-202.
- Veteli, T. O., K. Kuokkanen, R. Julkunen-Tiitto, H. Roininen, and J. Tahvanainen. 2002. Effects of elevated CO<sub>2</sub> and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology* 8:1240-1252.
- Viechtbauer, W. 2010. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software* 36:1-48.
- Vogel, C. S., P. S. Curtis, and R. B. Thomas. 1997. Growth and nitrogen accretion of dinitrogen-fixing *Alnus glutinosa* (L) Gaertn under elevated carbon dioxide. *Plant Ecology* 130:63-70.
- Wan, S. Q., R. J. Norby, K. S. Pregitzer, J. Ledford, and E. G. O'Neill. 2004. CO<sub>2</sub> enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. *New Phytologist* 162:437-446.
- Wang, D., S. A. Heckathorn, X. Z. Wang, and S. M. Philpott. 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO<sub>2</sub>. *Oecologia* 169:1-13.



- Watanabe, M., Q. Mao, E. Novriyanti, K. Kita, K. Takagi, F. Satoh, and T. Koike. 2013. Elevated CO<sub>2</sub> enhances the growth of hybrid larch F1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) seedlings and changes its biomass allocation. *Trees* 27:1647-1655.
- Wayne, P. M., E. G. Reekie, and F. A. Bazzaz. 1998. Elevated CO<sub>2</sub> ameliorates birch response to high temperature and frost stress: implications for modeling climate-induced geographic range shifts. *Oecologia* 114:335-342.
- Zaehle, S., P. Ciais, A. D. Friend, and V. Prieur. 2011. Carbon benefits of anthropogenic reactive nitrogen offset by nitrous oxide emissions. *Nature Geosci* 4:601-605.
- Zaehle, S., A. D. Friend, P. Friedlingstein, F. Dentener, P. Peylin, and M. Schulz. 2010. Carbon and nitrogen cycle dynamics in the O-CN land surface model: 2. Role of the nitrogen cycle in the historical terrestrial carbon balance. *Global Biogeochemical Cycles* 24:GB1006.
- Zak, D. R., K. S. Pregitzer, P. S. Curtis, J. A. Teeri, R. Fogel, and D. L. Randlett. 1993. Elevated Atmospheric CO<sub>2</sub> and Feedback between Carbon and Nitrogen Cycles. *Plant and Soil* 151:105-117.
- Zak, D. R., K. S. Pregitzer, P. S. Curtis, C. S. Vogel, W. E. Holmes, and J. Lussenhop. 2000. Atmospheric CO<sub>2</sub>, soil-N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. *Ecological Applications* 10:34-46

## CHAPTER 3

### **Do C4 plants exhibit optimal stomatal behaviour? A test with congeneric C3 and C4 species**

**Summary** A fundamental resource trade-off controlled by stomata of plants is the acquisition of CO<sub>2</sub> as photosynthetic substrate versus water loss through transpiration. The theory of optimal stomatal behaviour says that stomata of plants should act to maximize carbon gain (photosynthesis,  $A$ ) while minimizing water loss (transpiration,  $E$ ). That is, the optimal stomatal behaviour would be to maximise the integrated sum of  $(A - \lambda E)$ , where  $\lambda$  (mol C mol<sup>-1</sup> H<sub>2</sub>O) represents the marginal carbon cost of water. A recent model developed by Medlyn et al. (2011) combines the optimal theory and empirical approach to capture stomatal responses for the C3 plants. However, the model has not been tested for the alternative photosynthetic pathway occurring in C4 plants. Since C4 plants respond differently in terms of saturated photosynthetic rates to CO<sub>2</sub> concentrations ( $C_a$ ), we can expect different stomatal responses from them. In the study, congeneric species of C3 and C4 types were used to measure their responses to changes in  $C_a$  and vapour pressure deficit ( $D$ ). The unified model by Medlyn et al. (2011) was used to predict the plant responses: this model represents the value of  $\lambda$  through a parameter called  $g_1$ . The results showed that the C4 plants followed the same stomatal behaviour predicted for C3 plants. This suggests that stomatal responses of C4 plants match C3 responses while attaining maximum rates of photosynthetic activity. Hence, the unified model by Medlyn et al. (2011) is recommended as a framework for interpreting C4 stomatal responses to environmental factors.

### **3.1 Introduction**

Exchange of CO<sub>2</sub> and water vapour occurs through small apertures in the leaves of plants called stomata. Stomata of plants facilitate one of the most important physiological trade-offs because they control the acquisition of CO<sub>2</sub> as a photosynthetic substrate whilst limiting transpiration to maintain a favourable water potential in leaf tissues, (Cowan 1982, Zeiger 1987). Stomatal apertures are sensitive to multiple environmental influences such as light, intercellular CO<sub>2</sub> partial pressure ( $C_i$ ), humidity, soil water potential, and temperature (Zeiger 1983). The physiological mechanisms controlling the response of stomata to environmental conditions are complex and not fully understood. To describe stomatal

response to environmental and physiological factors, empirical models are often used which are based on statistical correlations between environmental or internal factors (Ball et al. 1987, Leuning 1990b, Collatz et al. 1991). Such models are commonly used to predict canopy photosynthesis and plant water use under varying  $C_a$  and temperatures. Some models concentrate at leaf level (Ball et al. 1987, Leuning 1990b, Leuning et al. 1995) while others are scaled from the leaf up to canopies, landscapes or the globe (Jensen et al. 1992, McMurtrie et al. 1992, Sellers et al. 1992, Gedney et al. 2006, Cao et al. 2010).

An alternative approach to predicting stomatal behaviour is to use optimisation theory. Optimisation theory hypothesizes that plants will tend to gain carbon most economically with respect to water loss. Plants regulate  $g_s$  to make optimal use of a finite, fixed water supply. This theory results in the prediction that the marginal gain of assimilation rate (A) with respect to transpiration rate (E) is uniform and constant (Cowan and Farquhar 1977). Mathematically, this can be written as: plants maximise

$$A - \lambda E \quad (1)$$

Resulting in the prediction that

$$\frac{\partial A}{\partial E} = \lambda \quad (2)$$

where A is the assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and E denotes water loss through transpiration ( $\text{mol m}^{-2} \text{s}^{-1}$ ). The parameter  $\lambda$  ( $\text{mol C mol}^{-1} \text{H}_2\text{O}$ ) denotes the marginal carbon cost of water supplied to leaves. Since the theory was first proposed, a number of authors have applied this theory to describe stomatal behaviour (Hari et al. 1986, Lloyd 1991, Thomas et al. 1999, Arneth et al. 2002, Katul et al. 2010). A major drawback in implementing this theory, however, has been the need to estimate a value for the parameter  $\lambda$ . Secondly, many model implementations fail to capture stomatal responses with increase in  $C_a$  (Lloyd et al. 2002).

Recently Medlyn et al. (2011) proposed a novel implementation of the optimal stomatal theory. They reconciled the two approaches (empirical and optimal) and presented a model of stomatal conductance ( $g_s$ ) derived from optimal theory. This unified stomatal model is similar to existing empirical models (Collatz et al. 1991, Leuning et al. 1995) and also provides a theoretical interpretation for model parameter values. Furthermore, it can be readily parameterized with field data like the empirical models. The mathematical form of the model is:

$$g_s = g_o + 1.6 \left( 1 + \frac{g_o}{\sqrt{D}} \right) \frac{A}{C_a} \quad (3)$$

where  $g_s$  is stomatal conductance,  $g_o$  is the intercept or residual conductance, A is the assimilation rate,  $C_a$  is the atmospheric  $\text{CO}_2$  concentration, D is the vapour pressure deficit

and  $g_1$  represents the slope of the relationship between  $g_s$  and the combination of terms  $A/C_a \sqrt{D}$ . The parameter  $g_1$  has been interpreted as proportional to the marginal water cost of carbon gain ( $\lambda$ ) (Medlyn et al. 2011).

One of the important assumptions made in the derivation of equation (3) (the unified model) is that it is based on the limitation to photosynthesis which occurs in C3 plants. Typically, photosynthesis is modelled as being the most limiting of two processes, RuBP regeneration or Rubisco activity (Farquhar and von Caemmerer 1982). In the derivation of equation (3), it is assumed that stomata of C3 plants optimize for the RuBP-regeneration limited reaction rather than the Rubisco limited reaction. By assuming RuBP regeneration is limiting to photosynthesis, the model accurately predicts the stomatal conductance response to increasing  $C_a$  (Medlyn et al. 2013). The  $g_1$  parameter value has been found to remain unchanged between  $CO_2$  treatments, indicating no acclimation of stomatal conductance to  $CO_2$  enrichment (Barton et al. 2012, De Kauwe et al. 2013). The model has been tested on various C3 woody species (Medlyn et al. 2011, Herault et al. 2013) and seems to capture the plant responses well. The model, however, has only been derived and tested for C3 species, whereas some plants possess an alternate C4 photosynthetic pathway. It is unclear if the optimisation theory can predict stomatal responses for these species. Other approaches, however, have been used for predicting  $g_s$  responses to  $CO_2$  for C3 (Hari et al. 1986, Lloyd 1991, Katul et al. 2010) and C4 species (Manzoni et al. 2011, Way et al. 2014).

The C4 photosynthesis pathway is an elaborated addition to the C3 photosynthetic pathway and is thought to have originated from ancestral C3 plants via a series of anatomical and physiological adaptations to high light intensities, high temperatures, and dryness (Sage and Kubien 2003). The key initial step in the evolution of C4 photosynthesis is thought to be the development of the photo-respiratory  $CO_2$  pump. Photo-respiration acts as the evolutionary link leading from C3 photosynthesis to the intermediate stages where the PCA (Primary Carbon assimilation) cycle can begin to develop (Sage 2001). Moreover, the partial closure of stomata to conserve water in arid and saline soils or dry atmospheric conditions has been hypothesized to select for the C4 pathway via indirect effects on photosynthetic efficiency (Ehleringer et al., 1993). Thus, reduced stomatal aperture not only restricts the  $CO_2$  supply to photosynthesis but also decreases transpiration, thereby reducing latent heat loss and raising leaf temperature. Both effects increase photorespiration, depressing the efficiency of C3 photosynthesis, and favouring the C4 type.

In all plants  $CO_2$  is fixed by the enzyme Rubisco. In C4 photosynthesis the Rubisco enzyme reaction is compartmentalised differently. Atmospheric  $CO_2$  is first fixed into C4

acids in mesophyll cells by the enzyme PEPC (phosphoenolpyruvate carboxylase) and C4 acids donate CO<sub>2</sub> to Rubisco in the bundle sheath cells. Here, CO<sub>2</sub> is released by one of three different decarboxylating enzymes, which define the three basic biochemical sub-types of C4 photosynthesis, NADP-dependent malic enzyme (NADP-ME), NAD-dependent ME (NAD-ME), and PEP carboxykinase (PEPCK). The functioning of mesophyll and bundle-sheath cells is well-co-ordinated which produces a high CO<sub>2</sub> concentration in the bundle sheath, effectively inhibiting photorespiration.

The role of bundle sheath cells is believed to reduce CO<sub>2</sub> leakage (Körner et al. 2003) however, the C4 cycle is prone to leakiness as some of the concentrated CO<sub>2</sub> diffuses back from the site of C4 acid decarboxylation. Hattersley et al. (1982) hypothesized that the leakiness of the bundle sheath cell wall is related to each sub-pathway type within C4. The NADP-ME subtype is purported to have the tightest bundle sheath conductance, NAD-ME the leakiest, and the PCK group is intermediate (Hatch et al. 1995). Due to this difference in leakiness, different C4 subtypes are thought to respond differently to C<sub>a</sub> (LeCain and Morgan 1998). At relatively low C<sub>i</sub>, C4 plants typically exhibit A/C<sub>i</sub> curve of steep initial slope with a plateau of early saturation. However, a number of studies have found that not all of the C4 species have photosynthesis that is saturated at present C<sub>a</sub> (Wong 1979, Morgan et al. 1994, Watling and Press 1997, Ziska and Bunce 1997, LeCain and Morgan 1998, Wand et al. 1999, Ziska et al. 1999).

In the last two decades, a large body of literature has been published on C3 species, while research into the response of C4 species has received much less attention. It is commonly assumed that because they possess a natural biochemical CO<sub>2</sub> concentrating mechanism, C4 plants will show no or limited growth response to rising C<sub>a</sub> (Hatch 1987) which ensures higher carbon assimilation rate and dry matter production, when stomata closure reduces CO<sub>2</sub> supply (Larcher 1995). Consequently, low stomatal conductance allows low transpiration rate and is expected to increase water use efficiency in C4 plants (Zhang and Kirkham 1995, Sage 2004, Tilman et al. 2006, Edwards et al. 2010, Edwards and Smith 2010).

The differences in physiological responses of C4 plants has led to the development of specific models describing C4 photosynthesis and their stomatal responses to environmental conditions. For example, one of the widely used coupled Photosynthesis-Stomatal conductance model derived by Collatz et al. (1992) for C4 species predicted a linear relationship between g<sub>s</sub> and A, leaf surface CO<sub>2</sub> and relative humidity. They found that the slope of the model was less than half of the values reported for C3 species. The results were

similar to previous studies for a number of C4 species as reported by Norman and Polley (1989) and Ball (1988) and for C3 species as reported by Ball (1988) and Leuning (1990a).

Moreover, it has been shown that the phylogenetic diversity underlying C4 photosynthesis is an important factor in understanding its functional consequences (Taylor et al. 2010, 2012). Taylor et al. (2010), comparing C3 and C4 grasses by sampling in a phylogenetic order, showed that it was a consistent characteristic of C4 species to conserve water through reduced  $g_s$ . They found significant phylogenetic patterns in  $g_s$  attributed to differences in stomatal pore size and density. A shift towards smaller stomatal size was found at a given density in C4 species. Also a higher relationship of greater stomata size and density was found in wetter habitat as compared to dry arid environment. Their work shows that C4 species have the characteristic of adapting themselves to the environment by various biochemical means (Sage 2004) and anatomical means such as stomatal patterning.

In the biochemical process of C3 photosynthesis, the initial slope of the  $\text{CO}_2$  response curve at light saturation, generally reflects the limitation of Rubisco enzyme. Whereas in C4 photosynthesis, the initial  $A/C_i$  slope is modelled to largely reflect the activity of PEPCase enzyme (von Caemmerer and Furbank 1999, von Caemmerer 2000), and the  $\text{CO}_2$  saturated plateau is modelled to reflect the limitation of the capacity of either Rubisco enzyme, RuBP regeneration, or PEP regeneration (von Caemmerer and Furbank 1999, von Caemmerer 2000, Sage 2002). Moreover, the high  $\text{CO}_2$  concentration attained in bundle sheath cells of C4 effectively reduces the reaction with  $\text{O}_2$ . As a result of this difference between their photorespiratory reduction responses, C4 photosynthesis differs in several ways from C3 photosynthesis. Firstly, there is a lower  $\text{CO}_2$  compensation point, which depends on the  $\text{O}_2$  concentration (Björkman et al. 1971). Secondly, since photorespiration is greatly suppressed, the C4 pathway does not have any inhibition of photosynthesis by  $\text{O}_2$  (Laik and Edwards 1997). Thirdly, the quantum yield for photosynthesis is not affected by  $\text{O}_2$ ,  $\text{CO}_2$  or temperature (Collatz et al. 1992, Ehleringer et al. 1997). Thus, at leaf level, C4 photosynthetic pathway has an advantage over C3 pathway which consists of several biochemical (Ghannoum 2009) and structural adaptations (Taylor et al. 2012). Due to these differences in photosynthesis limitation reactions and physiological traits, the optimal stomatal model described by Medlyn et al. (2011), needs to be tested for the C4 plant responses to elevated  $\text{CO}_2$  ( $eC_a$ ).

Therefore, the objectives of the current study were to analyse whether the optimal model can describe stomatal conductance in C4 plants. To do so, responses of stomatal conductance were measured to varying  $\text{CO}_2$  and D in a range of C3 and C4 species. Species

were selected from genera *Flaveria* and *Panicum* because these genera have species with C3, C3-C4 intermediate and C4 photosynthetic pathways (Hatch 1987). So there is advantage of examining the *Panicum* and *Flaveria* genera more closely as these genera represent a range of naturally occurring variants of photosynthetic types. Both genera contain C3-C4 intermediate types in addition to species with well-defined C3 and C4 traits. *Flaveria* is a dicot genus whereas *Panicum* represents the monocots. Moreover, both genera contain all three C4 subtypes (NADP-ME, NAD-ME and PEP-CK). However, to represent NADP-ME C4 type in monocot in the present study, *Paspalum* species was selected. The main focus was to compare differences among congeneric species.

The analysis was based on the idea that since C4 plants have different photosynthetic pathway and saturate at lower CO<sub>2</sub> levels, they would adjust their stomatal conductance to optimise their water use in a similar manner to the C3 species. To test this hypothesis, I used the optimal stomatal model to predict stomatal behaviour for C4 plants under varying CO<sub>2</sub> and VPD. The ultimate goals being (a) to understand how stomatal behaviour has evolved in C4 plants and (b) to enable better prediction of C4 stomatal responses to environmental factors.

## 3.2 Material and methods

### 3.2.1 Plant material

Three C3 and four C4 species were selected for the experiment (Table 3.1). Seeds were acquired from University of Western Sydney, Richmond, NSW. C3 species were; *Steinchisma laxa* (synonym *Panicum laxa*), *Panicum bisulcatum* and *Flaveria pringlei*. C4 species were; *Panicum maximum*, *Panicum coloratum*, *Paspalum dilatatum* and *Flaveria bidentis*. The species photosynthetic sub-pathway types along with functional-type are shown in table 3.1. Note that *P. dilatatum* was used to represent C4 NADP-ME monocot species. Seeds were germinated in a potting mix tray for four weeks. After four weeks, seedlings were planted in 10 litre pots with organic soil mix. The soil mixture contained slow release fertiliser, providing sufficient nutrients for a month of plant growth. After one month, plants were regularly fertilized with 100g/week Nitrosol solution which contains Nitrogen, Phosphorus and Potassium in the amount of 8% – 2% – 5.8%.

Only plants of *F. pringlei* were propagated from shoot cuttings which were later planted in 10 litre pots. There were five replicates of each species so thirty five pots in total

were used. Seedlings were grown in a naturally lit glasshouse for sixteen weeks with ambient CO<sub>2</sub> concentration. The temperature in the glasshouse was controlled at 27/21 °C for day and night cycle. The incident photon flux density was decreased to 1300  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  due to the glass. When plants were four months old, gas exchange measurements were done, starting from first week of December 2012 to late January 2013. Five to six replicates of species pots were selected at random and were moved to an artificially illuminated growth chamber.

**Table 3-1:** Names of the species used to measure gas-exchange data. Three C3 and four C4 species were used. C4 species had sub-photosynthetic pathways as shown. Each C3 species is listed against its congener C4 species.

C3 species			C4 Species			
Species Name	Pathway Type	Functional Type	Species Name	Pathway Type	Sub-Pathway Type	Functional Type
<i>Flaveria pringlei</i>	C3	Dicot	<i>Flaveria bidentis</i>	C4	NADP-ME	Dicot
<i>Panicum bisulcatum</i>	C3	Monocot	<i>Panicum coloratum</i>	C4	NAD-ME	Monocot
<i>Steinchisma laxa</i>	C3	Monocot	<i>Panicum maximum</i>	C4	PCK	Monocot
			<i>Paspalum dilatatum</i>	C4	NADP-ME	Monocot

Measurements were taken after one day when plants were well adjusted to chamber environment. The temperature was controlled similar to glasshouse whereas photosynthetic photon flux density (PPFD) ranged from the top of the chamber from 800  $\mu\text{mol m}^{-2}\text{s}^{-1}$  to 300  $\mu\text{mol m}^{-2}\text{s}^{-1}$  close to the bottom of the chamber. PPFD at plant height was about 700  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Relative humidity or D (vapour pressure deficit) was not controlled in the glasshouse or in the growth chamber (ranged from *c.* 1 to 1.7 kPa). Measurements were taken during a four week period, during which some plants got infected with aphids. Aphid control spray was used twice during the time and no measurements were taken until the plants had shown full recovery from the pest infection. The experiment took place at Macquarie University, Sydney, Australia.

### 3.2.2 Gas exchange

Steady-state leaf gas-exchange was measured at saturating irradiance with an open gas-exchange system (LI-6400XT; Li-Cor Inc., Lincoln, NE, USA) between 0900 and 1500 h Australian Eastern Daylight Savings Time. The leaf from second whorl was sampled on three to four randomly selected plants at four treatment CO<sub>2</sub> concentrations (200, 380, 550 and 800  $\mu\text{mol mol}^{-1}$ ). Measurements were done at saturating light levels of 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . A Peltier cooling module maintained leaf temperatures at approximately ambient conditions (i.e.



at 27 °C). For each measuring day, leaf-to-air vapour pressure deficit (D) was maintained at levels 1, 1.5, 2 or 2.5 kPa for each species in the leaf cuvette. Hence, only CO<sub>2</sub> concentration was altered during the day with a fixed D. The CO<sub>2</sub> concentration supplied to the leaf cuvette was changed in the sequence: 380, 200, 550, and 800 µmol mol<sup>-1</sup>, with over 1 hour equilibration at each concentration. High levels of D were maintained by using the desiccant, Drierite inside LI-6400XT, whereas to maintain low levels of D, a bubbler was attached to the inlet valve of LI-6400XT. Leaf area was calculated from leaf dimensions.

To examine stomatal responses to changing CO<sub>2</sub> concentration, I measured the response of g<sub>s</sub> to a manipulation of CO<sub>2</sub> concentration within the leaf cuvette. Once clamped in the cuvette, leaves were exposed to the light, D, and temperature levels described above and the respective atmospheric CO<sub>2</sub> concentration. Near ambient CO<sub>2</sub> concentration (380 µmol mol<sup>-1</sup>) were adjusted by using soda lime scrubber within the LI-600XT. After steady-state conditions were achieved, the first measurement was taken and CO<sub>2</sub> concentration was then decreased to 200 µmol mol<sup>-1</sup>. Stomatal conductance was recorded after steady-state conditions were re-established. CO<sub>2</sub> concentrations were then increased to 550 µmol mol<sup>-1</sup> and 800 µmol mol<sup>-1</sup> till the steady-state conditions were attained at each CO<sub>2</sub> level. Steady-state was determined when the coefficient of variation of change in each measurement was <0.05% (generally after 40min to 1 hour). The response of net photosynthesis (A) was recorded simultaneously with g<sub>s</sub>.

### 3.2.3 C3 and C4 models

Two different approaches for fitting C4 A–C<sub>i</sub> curves were used.

In the first approach, an empirical rectangular hyperbola function was fitted to the A–C<sub>i</sub> data:

$$A = \frac{A_{\max} C_i}{C_i + K_m} \quad (4)$$

Fitting this equation yields two parameters, A<sub>max</sub>, the maximum assimilation rate (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-2</sup>) and K<sub>m</sub>, the Michaelis–Menten constants. Assuming that equation (4) describes photosynthesis, the optimal stomatal behaviour can be derived. It gives a quadratic solution for the intercellular CO<sub>2</sub> concentration, C<sub>i</sub> (cf. Arneth et al. 2002):

$$a. C_i^2 + b. C_i + c = 0 \quad (5)$$

The coefficients a, b and c are given by:

$$a = K_m - L \quad (6)$$

$$b = -2C_a K_m \quad (7)$$

$$c = C_a K_m (C_a - L) \quad (8)$$

Where  $C_a$  is the ambient  $\text{CO}_2$  concentration and  $L$  denotes the term:

$$L = 1.6 \frac{D}{P} \lambda \quad (9)$$

where  $D$  is the leaf-to-air vapour pressure difference (kPa) and  $P$  is the atmospheric pressure (kPa).  $\lambda$  denotes the carbon cost of water ( $\mu\text{mol C mol}^{-1} \text{H}_2\text{O}$ ). This quadratic equation is fitted to data to obtain  $\lambda$  values. The optimal stomatal conductance  $g_s$  is calculated from the optimal  $C_i$  as:

$$g_s = \frac{1.6 A}{C_a - C_i} \quad (10)$$

In the second approach, the full theoretical model of Farquhar et al. (1980) and von Caemmerer (2000) for  $\text{C}_4$  photosynthesis was fit data to the  $A$ - $C_i$  responses to obtain three parameters,  $V_{\text{cmax}}$ , the maximum Rubisco carboxylation rate,  $V_{\text{pmax}}$ , the maximum PEP carboxylation rate, and bundle sheath conductance ( $g_{\text{bs}}$ ). We then solved the stomatal optimization model numerically to calculate optimal  $g_s$ , and fit the numerical solution to data to estimate values of  $\lambda$ .

Finally, the unified model from Medlyn et al. (2011) was also fit to data to obtain  $g_1$  values (Equation 3) using a non-linear least square fit to the data. The fit to the data was assessed in each case by calculating root-mean-square error (RMSE). For *Flaveria* species the highest  $D$  values (2.5 kPa) were omitted in calculating  $\lambda$ .

#### 3.2.4 Statistical analysis

$\text{C}_3$  and  $\text{C}_4$  model calculations were done using R-packages ‘GasExchangeR’ (Duursma 2012) and ‘plantecophys’ (Duursma 2014). All statistical analyses were performed with ‘R 3.1.0’ (R Development Core Team 2010, 2014).

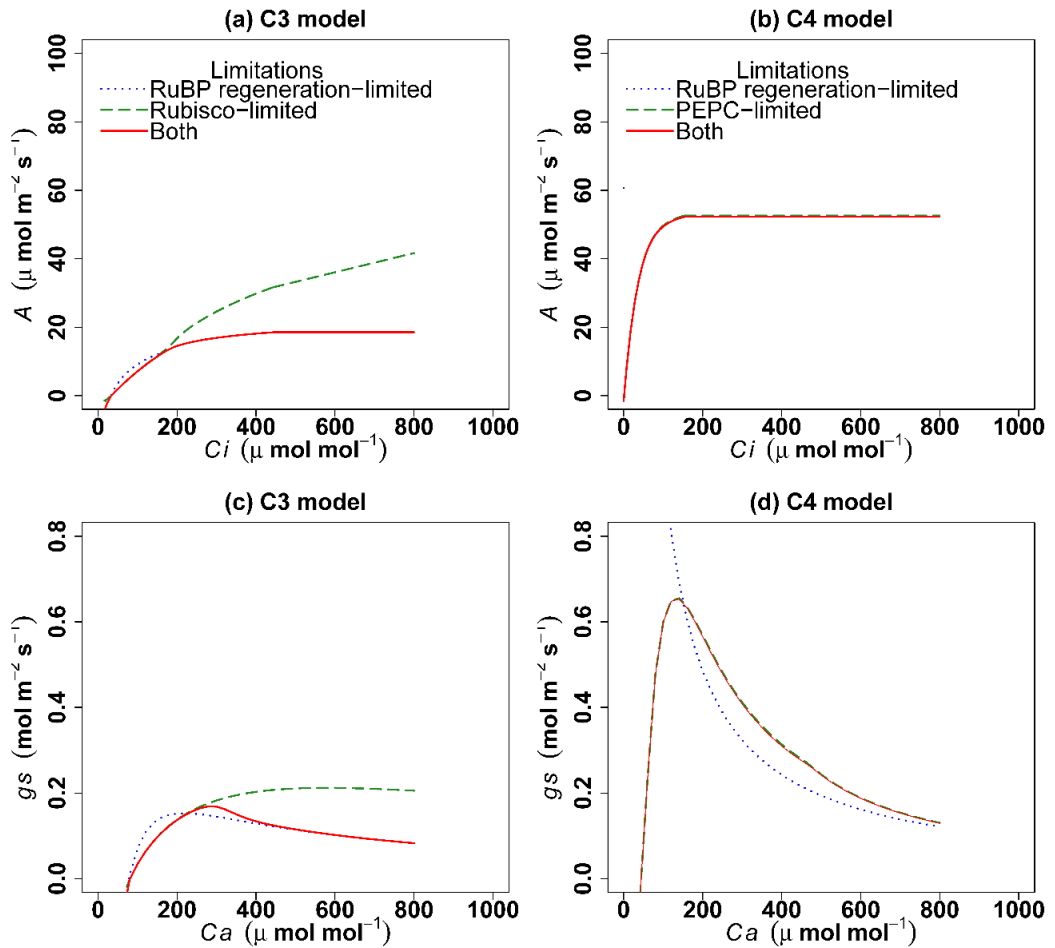
### 3.3 Results

#### 3.3.1 $\text{C}_3$ and $\text{C}_4$ model

Figures 3.1a and 3.1b show the characteristic modelled responses of  $\text{C}_3$  and  $\text{C}_4$  to  $C_a$  according to von Caemmerer (2000). In  $\text{C}_3$  plants, an increase in  $C_a$  leads to a rise in the intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (Figure 3.1a) which in turn leads to higher assimilation rates ( $A$ ). The initial slope of the curve depicts rise in  $A$  due to Rubisco limited reaction ( $V_{\text{cmax}}$ ), whereas at higher  $\text{CO}_2$ , plants follow the RuBP-regeneration limited reaction ( $J_{\text{max}}$ ). In  $\text{C}_4$  plants, the initial slope of the  $A/C_i$  curve is much steeper and photosynthesis becomes saturated by  $\text{CO}_2$  at a lower  $C_i$  (Figure 3.1b). The initial slope of the curve is proportional to

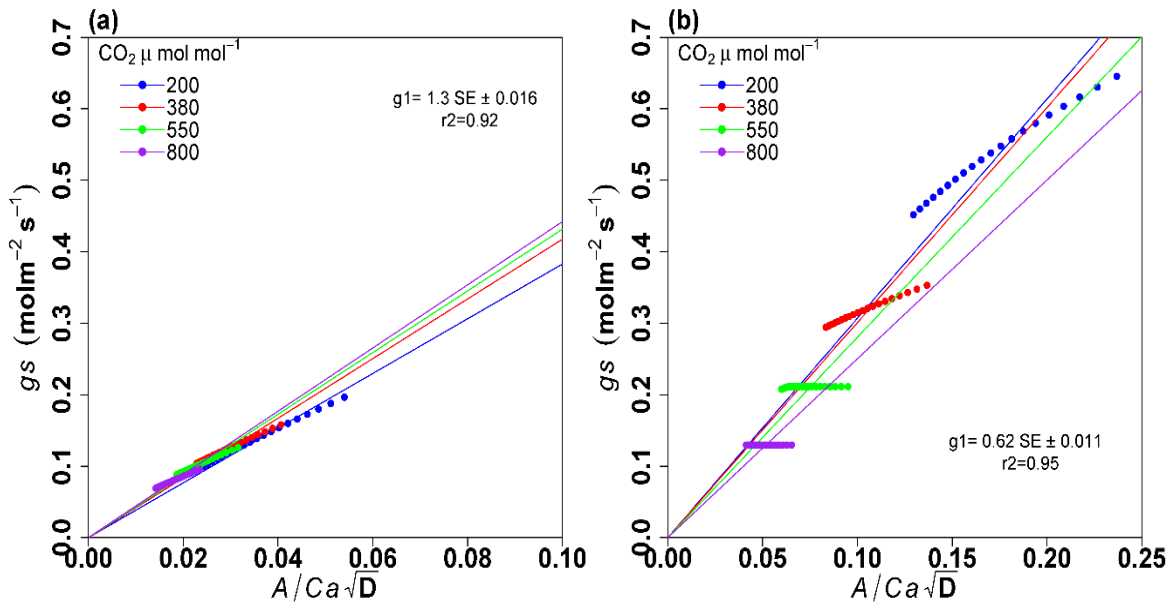
the PEPC ( $V_{pmax}$ ), whereas the saturated rate is proportional to Rubisco activity ( $V_{cmax}$ ), the rate of PEP regeneration ( $V_{pr}$ ), or an electron transport limitation ( $J_{max}$ ).

The resulting predicted optimal stomatal behaviour of C3 and C4 plants with increasing  $CO_2$  ( $C_a$ ) is shown in figure 3.1c and 3.1d. For C3 plants, optimal stomatal conductance is to open with rising  $C_a$  if Rubisco is limiting, and to close with rising  $C_a$  if RuBP-regeneration is limiting. It is known that stomata behave as if to optimise for RuBP-regeneration reaction (Figure 3.1c), i.e. stomata close with increasing  $CO_2$  (Medlyn et al. 2011, 2013). For C4 plants on the other hand, the optimal stomatal behaviour is to close with higher  $C_a$  under all limitations to photosynthesis (Figure 3.1d). The predicted rate of closure with rising  $CO_2$  is stronger in C4 plants than in C3 plants.



**Figure 3-1:** Modelled rate of  $CO_2$  assimilation as a function of intercellular  $CO_2$  concentration  $C_i$ , in (a) C3 and (b) C4 plants (from von Caemmerer 2000). The rubisco- limited (RuBP-saturated) or PEPC-limited rate of  $CO_2$  assimilation has a dashed green line extension at high  $CO_2$ . The electron-transport (RuBP-regeneration) limited rate has a dotted blue line extension at low  $CO_2$ . The solid red curve represents the minimum rate that is the rate of  $CO_2$  assimilation. The resulting predicted optimal  $g_s$  behaviour as a function of  $CO_2$  concentration ( $C_a$ ) is shown for (c) C3 and (d) C4 plants. Model parameters were:  $V_{cmax} = 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , LeafT=25°C,  $R_d = 1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ , lambda = 0.001 ( $\text{mol mol}^{-1}$ ).

Optimal stomatal behaviour modelled numerically for C3 and C4 plants was compared using the unified optimal model by Medlyn et al. (2011), using the same parameter estimates as used in experimental data protocol.  $g_s$  was visualised as a function of the term  $A/(C_a\sqrt{D})$  (Figure 3.2a & b). For C3 plants, the optimal stomatal behaviour follows closely the unified stomatal model (Figure 3.2 a), with linear relationship at  $CO_2$  concentrations of 200, 380, 550 and  $800 \mu\text{mol mol}^{-1}$ . Optimal stomatal behaviour for C4 plants appeared to follow an almost linear relationship at low  $CO_2$  concentration of  $200 \mu\text{mol mol}^{-1}$ , (Figure 3.2b) however at higher  $C_a$  the relationship starts to deviate from linear and the optimal stomatal behaviour model no longer applies. The scatter around the linear relationship is also much higher for C4 than C3 plants.

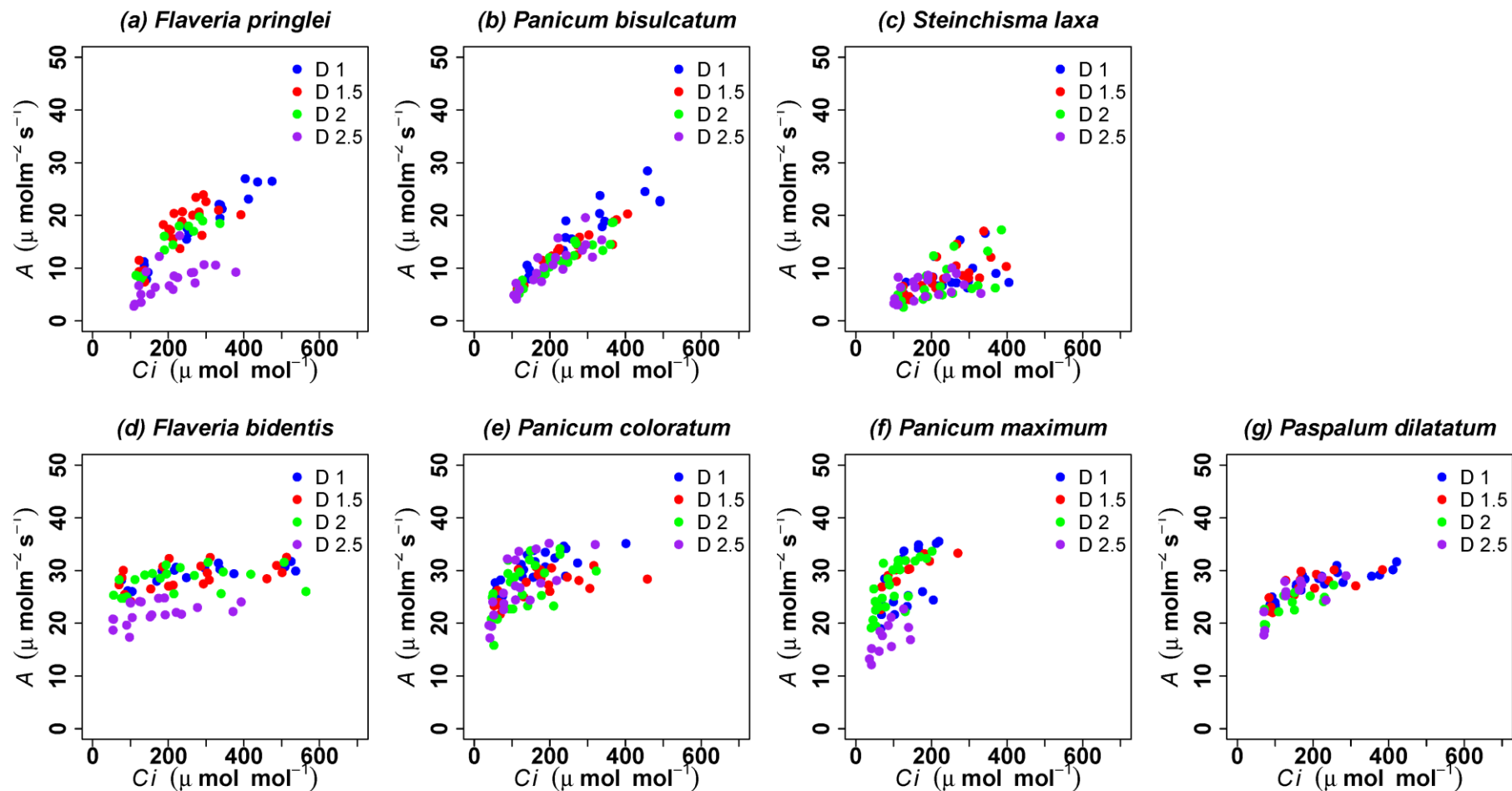


**Figure 3-2:** Modelled optimal  $g_s$  responses in (a) C3 and (b) C4 plants (from Medlyn et al. 2011). Stomatal conductance ( $g_s$ ) as a function of  $A/C_a\sqrt{D}$ . Dotted lines show the predicted values. Straight lines show linear fit to the model through zero intercept. Four  $CO_2$  concentrations ( $C_a$ ) were used (200, 380, 550 and  $800 \mu\text{mol mol}^{-1}$ ) with  $D$  ranging from 1 to 2.5 kPa. Slope or  $g_1$  was calculated by non-linear least square fit. Model parameters used were same as in Figure 3.1.

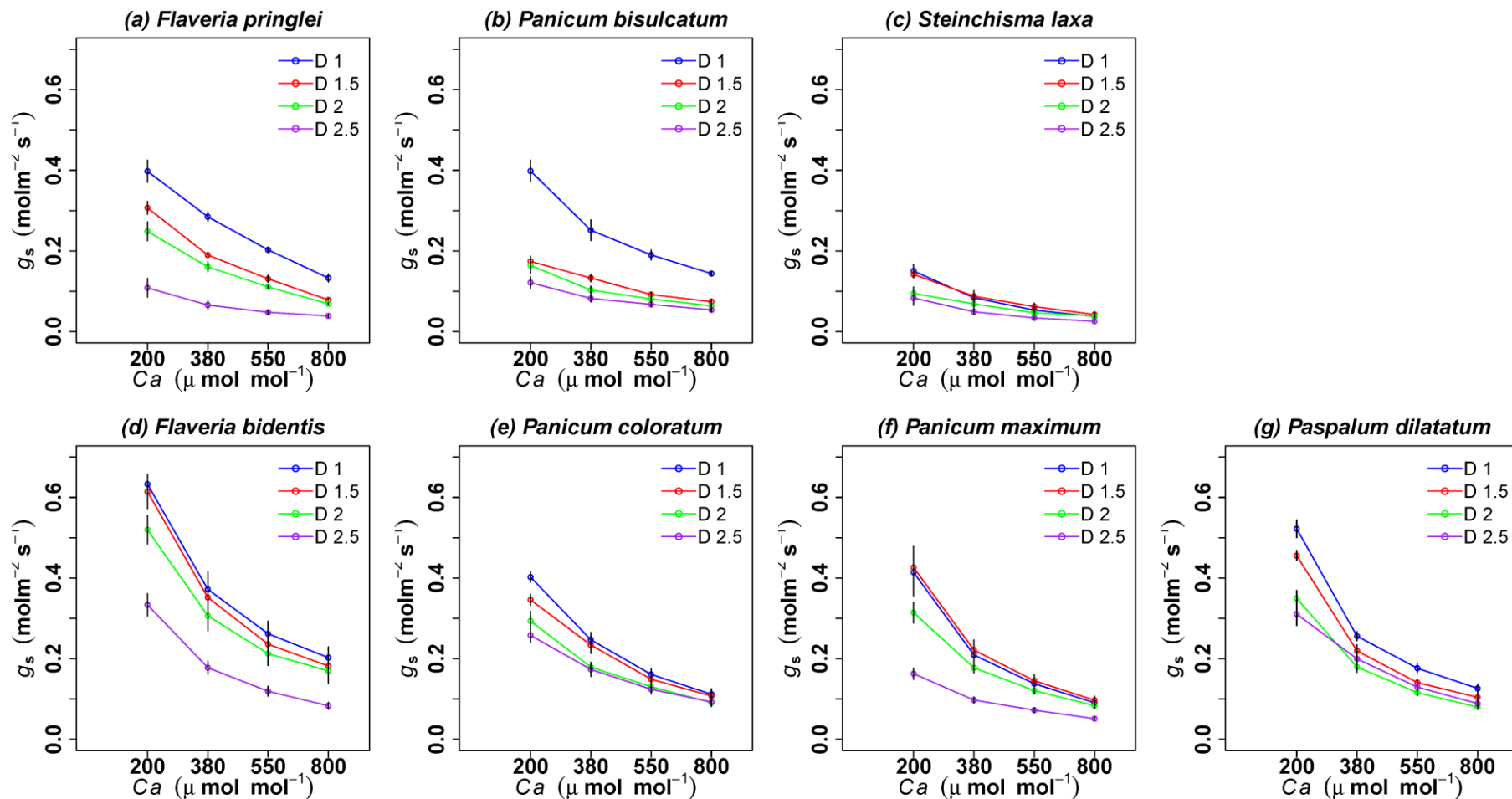
To calculate the optimal stomatal behaviour, it is first necessary to characterise the response of  $A$  to  $C_i$ . I did this by fitting two alternative equations to the  $A$  and  $C_i$  data, as described in the methods: firstly, a simple hyperbolic equation and secondly, the von Caemmerer et al. (2000) process-based photosynthesis model. The second equation is commonly fitted to measurements of  $A$  made over a wide range of  $C_i$  levels with the intention of obtaining accurate values for the key photosynthetic parameters  $V_{cmax}$  and  $V_{pmax}$ . However, the main objective in this study was not to obtain parameter values but rather to characterise the shape of the  $A$ - $C_i$  response over the relevant  $C_i$  range and hence there was no need to obtain measurements of photosynthesis over a wider range of  $C_i$  values. The fitted parameters  $A_{max}$  and  $K_m$  from the rectangular hyperbolic equation, and  $V_{cmax}$ ,  $V_{pmax}$  and  $g_{bs}$  from the von Caemmerer et al. (2000) model are given in table 3.2. For the von Caemmerer et al. (2000) model, *Panicum maximum* had higher  $V_{cmax}$ ,  $V_{pmax}$  and  $g_{bs}$  values than rest of the C4 species (Table 3.2).

**Table 3-2:** Rates of  $V_{cmax}$  and  $V_{pmax}$  as calculated from numeric solution of von Caemmerer (2000) C4 photosynthesis model to fitted data are listed. The numerical fit was also used to calculate  $g_{bs}$  values.  $A_{max}$  and  $K_m$  values as measured from hyperbolic solution to fitted data are shown. SE represents the standard error of the mean values.

C4 species											
Species name	Sub-pathway type	V <sub>cmax</sub> (num)	SE	V <sub>pmax</sub> (num)	SE	g <sub>bs</sub> (num)	SE	A <sub>max</sub> (hyph)	SE	K <sub>m</sub> (hyph)	SE
<i>Flaveria bidentis</i>	NADP-ME	31.32	(1.66)	86.79	(25.91)	0.0028	(0.0034)	30.75	(0.49)	11.56	(2.55)
<i>Panicum coloratum</i>	NAD-ME	33.79	(3.67)	75.13	(23.12)	0.0034	(0.0054)	34.32	(0.99)	26.2	(3.98)
<i>Panicum maximum</i>	PCK	47.46	(11.27)	94.56	(34.05)	0.023	(0.091)	38.88	(2.79)	48.6	(10.91)
<i>Paspalum dilatatum</i>	NADP-ME	33.37	(4.39)	55.03	(13.42)	0.0029	(0.0046)	32.83	(0.78)	38.96	(4.49)



**Figure 3-3:** CO<sub>2</sub> assimilation rate,  $A$ , as a function of intercellular CO<sub>2</sub> concentration  $C_i$ , for C3 (a-c) and C4 species (d-g) as measured from gas-exchange. Different dot colours correspond to different vapour pressure deficit  $D$  values used in the measurements (1, 1.5, 2 and 2.5 kPa). Gas-exchange measurements were made at CO<sub>2</sub> concentrations of 200, 380, 550 and 800  $\mu\text{mol mol}^{-1}$ , at temperature of 27°C.



**Figure 3-4:** Stomatal conductance  $g_s$ , as a function of CO<sub>2</sub> concentration  $C_a$ , for C3 (a-c) and C4 species (d-g) as measured from gas-exchange. Different line colours correspond to different vapour pressure deficit D values used in the measurements (1, 1.5, 2 and 2.5 kPa). Gas-exchange measurements were made at CO<sub>2</sub> concentrations of 200, 380, 550 and 800  $\mu\text{mol mol}^{-1}$ , at temperature of 27°C. Mean values are represented by dots. The black vertical lines represent standard error of the means of 3-5 replicates.

### 3.3.2 Measured gas exchange

Gas exchange measurements showed photosynthesis responses for C3 and C4 plants as predicted by the models. For C3 species (*F. pringlei*, *P. bisulcatum* and *S. laxa*) assimilation rates (A) continued to increase with increasing intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) (Figure 3.3 a, b & c). There was significant effects of vapour pressure deficit (D) and CO<sub>2</sub> on both photosynthesis and stomatal conductance in C3 and C4 plants (Table 3.3). In C4 species (*F. bidentis*, *P. coloratum*, *P. maximum* and *P. dilatatum*), A saturated at lower C<sub>i</sub> values and there were significant effect of D on the response curves (Figure 3.3 d, e, f & g) (Table 3.3). Low assimilation rates however, were noticeable for *F. bidentis* at high D of 2.5 kPa. There were no significant CO<sub>2</sub> x D interactions in C4 species (Table 3.3).

Stomatal conductance showed large non-linear decrease in response to C<sub>a</sub> and D for both C3 and C4 species (Figure 3.4) (Table 3.3). There were no significant interaction between CO<sub>2</sub> x D x Species for C4 plants (Table 3.3).

**Table 3-3:** Summary of the statistical significance of the effects of different CO<sub>2</sub> concentration C<sub>a</sub>, vapour pressure deficit D and Species used on assimilation rates A, and stomatal conductance g<sub>s</sub>. Data was log-transformed. For the gas exchange parameters 4 CO<sub>2</sub> concentrations were used (200, 380, 550 and 800 µmol mol<sup>-1</sup>) at 4 levels of D (1, 1.5, 2 and 2.5 kPa). 3 C3 and 4 C4 species were used. Significance level was analysed by 3-way analysis of variance (nested design) with CO<sub>2</sub>, D and species as independent variables; n.s., not significant (P > 0.05); \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001

	Main effects			Interactions			
	CO <sub>2</sub>	D	Species	CO <sub>2</sub> x D	CO <sub>2</sub> x Species	D x Species	CO <sub>2</sub> x D x Species
<b>C3</b>							
A µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>	***	***	***	ns	***	***	ns
g <sub>s</sub> mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>	***	***	***	**	***	***	*
<b>C4</b>							
A µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>	***	***	***	***	***	***	ns
g <sub>s</sub> mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>	***	***	***	ns	***	***	ns

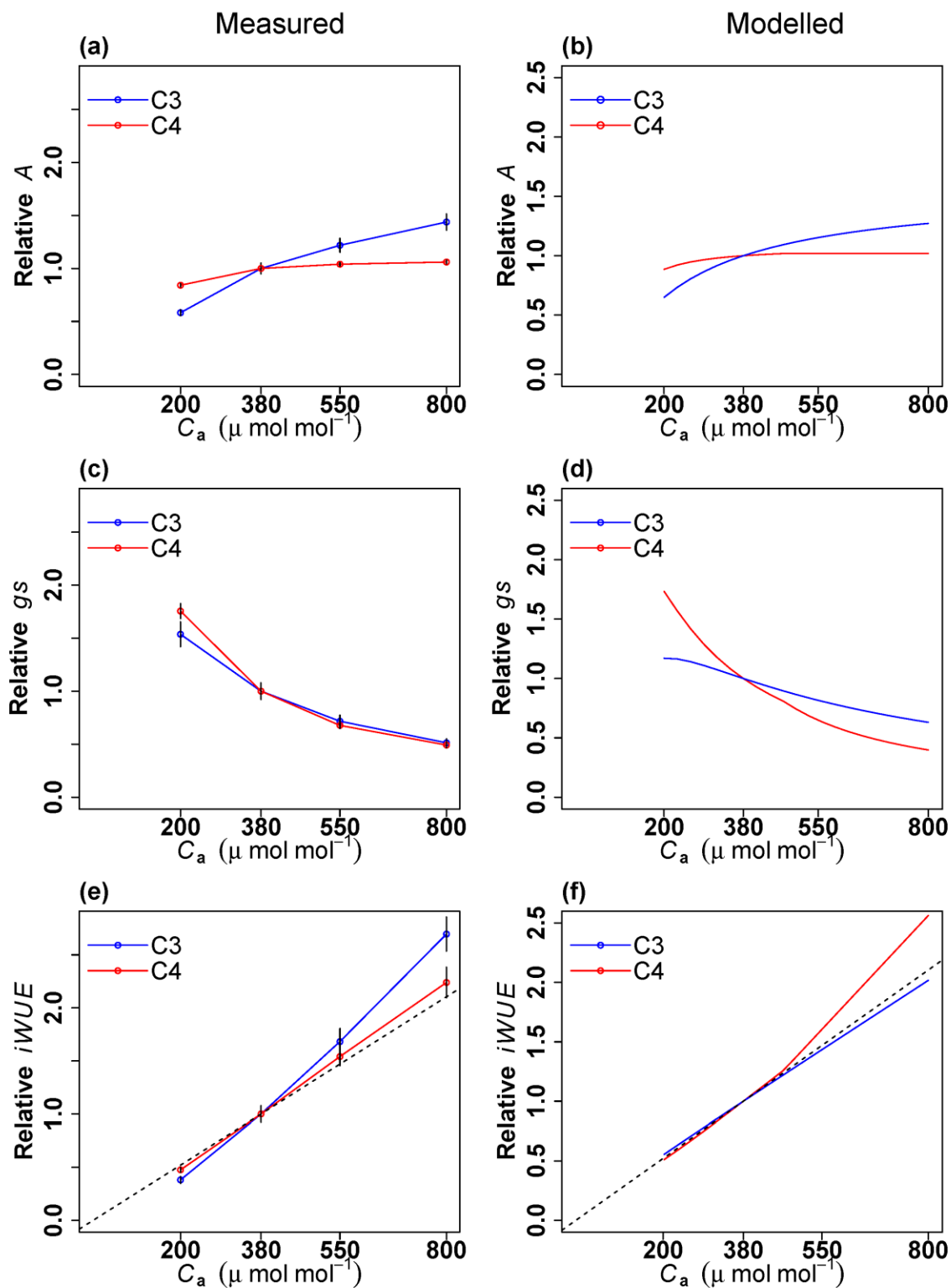
### 3.3.3 Comparison of model predictions to measurements

A comparison of relative responses averaged across D treatments for measured data showed that the C3 species had relatively higher assimilation rates (A) (Figure 3.5a) at high C<sub>a</sub> as compared to C4 species which showed a saturated response (Figure 3.5a). Comparing the measured A with the model predictions for C3 and C4 species (from von Caemmerer 2000) (Figure 3.5b) likewise showed increase in C3 assimilation rates but almost no increase for C4 species with increasing CO<sub>2</sub>.

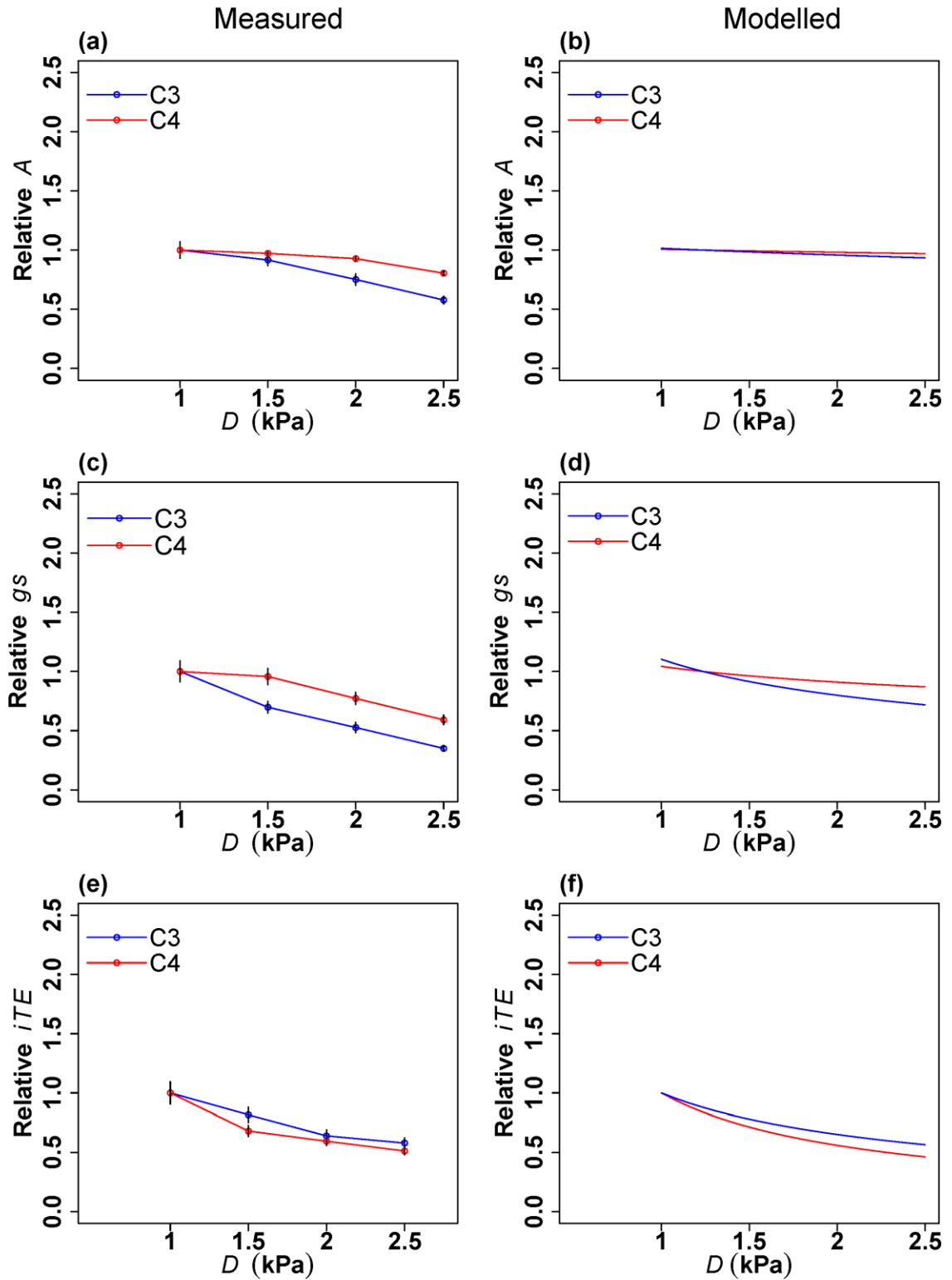


Relative  $g_s$  responses measured from gas-exchange data, showed non-linear decline with  $C_a$  for both C3 and C4 species at higher  $C_a$  (Figure 3.5c). However, model predictions (from von Caemmerer 2000) showed that  $g_s$  for C4 species are more sensitive than C3 species and declines more with increasing  $CO_2$  (Figure 3.5d). As a result of higher assimilation rates in C3 species, higher intrinsic water use efficiency ( $iWUE = A/g_s$ ) was observed as compared to C4 species (Figure 3.5e). This contradicts the model predictions that C4 species due to their relatively sensitive  $g_s$  will show higher  $iWUE$  at higher  $C_a$  (Figure 3.5f).

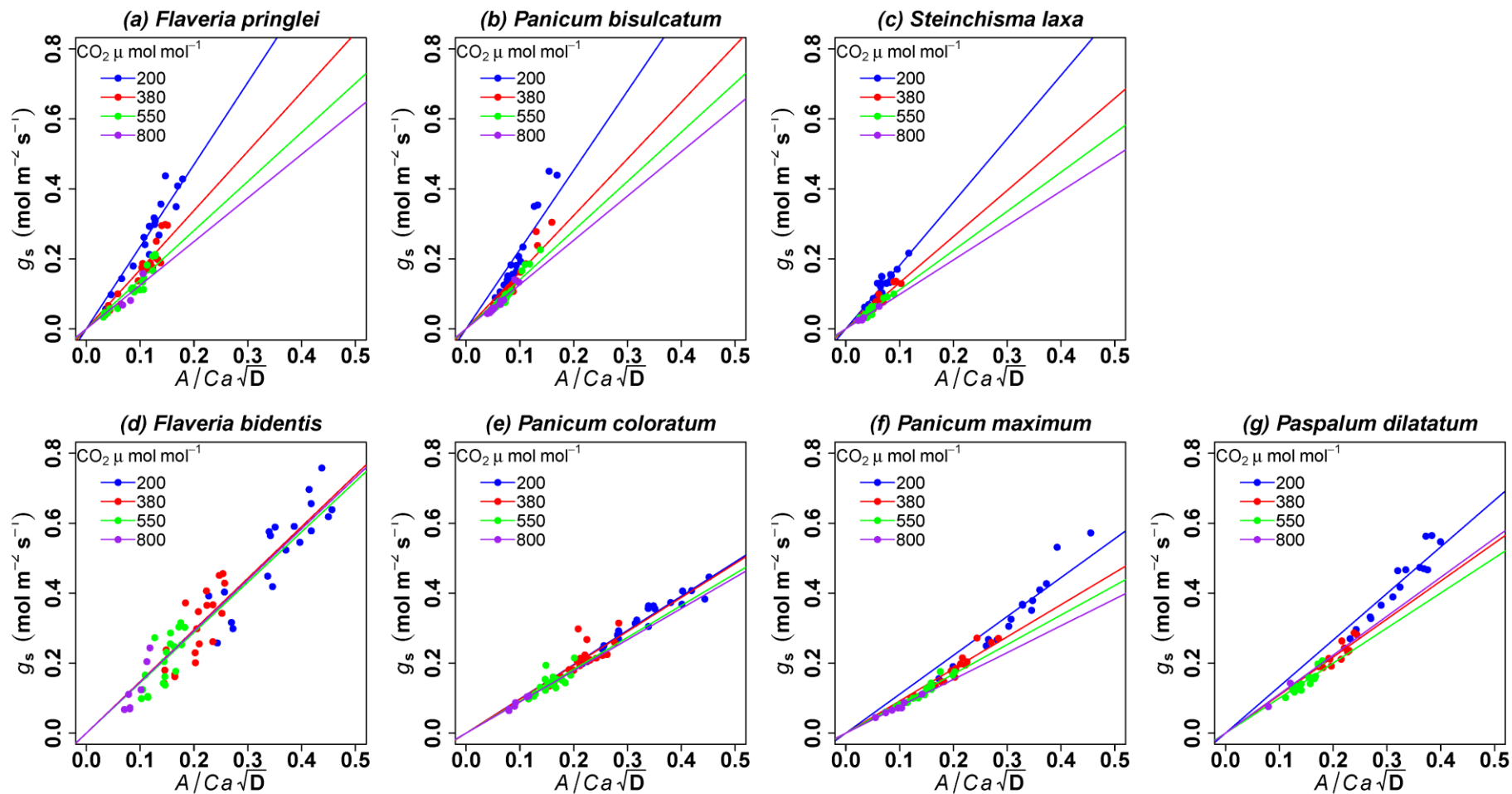
Both photosynthesis and stomatal conductance of C3 species were more responsive to leaf-to-air vapour pressure difference than those of C4 species (Figure 3.6).  $A$  declined by approximately 20% in C4 species and by approximately 43% in C3 species with increasing  $D$  (Figure 3.6a) was observed. The photosynthesis model however, predicted no difference in responses for C3 and C4 species (Figure 3.6b). Relative  $g_s$  declined non-linearly by approximately 65% with increasing  $D$  in the C3 species, whereas relative  $g_s$  declined by 41% in the C4 species (Figure 3.6c). Model predictions for  $g_s$  were similar for both C3 and C4 species (Figure 3.6d). Despite these differences in stomatal sensitivity to  $D$ , the accompanying decline in  $iTE (= A/E)$  was rather similar between the species (Figure 3.6e). The  $iTE$  values are analogous to the predicted photosynthesis model values (Figure 3.6f).



**Figure 3-5:** A comparison of the means ( $\pm 1$  SE) relative response of A (a),  $g_s$  (b) and  $iWUE$  ( $= A / g_s$ ) (c) to ambient CO<sub>2</sub> concentration ( $C_a$ ) in C3 and C4 species pooled across D treatments (1, 1.5, 2 and 2.5 kPa). Gas-exchange measurements were made at CO<sub>2</sub> concentrations of 200, 380, 550 and 800  $\mu\text{mol mol}^{-1}$ , at temperature of 27°C. Mean values are represented by dots. The black vertical lines represent standard error of the means of 5 replicates  $\times$  no. of species. Numeric photosynthesis model of von Caemmerer (2000) was used to predict C3 and C4 responses. Model parameters were:  $V_{\text{cmax}} = 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $\text{LeafT} = 27^\circ\text{C}$ ,  $R_d = 1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $\lambda = 0.001 (\text{mol mol}^{-1})$ .



**Figure 3-6:** A comparison of the means ( $\pm 1$  SE) relative response of A (a),  $g_s$  (b) and  $iTE$  ( $= A/E$ ) (c) to vapour pressure deficit (D) in C3 and C4 species pooled across  $CO_2$  treatments (200, 380, 550 and  $800 \mu mol mol^{-1}$ ). Gas-exchange measurements were made at D values of 1, 1.5, 2 and 2.5 kPa, at temperature of  $27^\circ C$ . Mean values are represented by dots. The black vertical lines represent standard error of the means of 5 replicates  $\times$  no. of species. Numeric photosynthesis model of von Caemmerer (2000) was used to predict C3 and C4 responses. Model parameters were:  $V_{cmax} = 50 \mu mol m^{-2} s^{-1}$ ,  $LeafT = 27^\circ C$ ,  $R_d = 1.5 \mu mol m^{-2} s^{-1}$ ,  $\lambda = 0.001 (mol mol^{-1})$ .



**Figure 3-7:** Relationship between  $g_s$  and the terms  $A/Ca\sqrt{D}$  from unified model of Medlyn et al. (2011) for C3 (a-c) and C4 (d-g) species. Each dot represents single gas-exchange measurement. Different colours correspond to different  $\text{CO}_2$  concentrations (see legend). Coloured lines represent linear regression fit to the data through zero intercept. Gas-exchange measurements were made at  $\text{CO}_2$  concentrations of 200, 380, 550 and  $800 \mu\text{mol mol}^{-1}$  with four levels of VPD 1, 1.5, 2 and 2.5 kPa, at temperature of  $27^\circ\text{C}$ . For slope parameter  $g_1$  refer to summary table (3.4)

**Table 3-4:** Summary table for figure (3.7) for C3 and C4 species at different CO<sub>2</sub> concentrations C<sub>a</sub>. Linear regression fit through zero was used to calculate slope of the data with resulting r-squared values. g<sub>1</sub> values were calculated by fitting non-linear least squares (nls) to the unified model by Medlyn et al. (2011). Root-mean-square error (RMSE) was calculated for the measured and predicted values.

Photosynthetic pathway	Sub-pathway	Seed type	Species	CO <sub>2</sub>	slope (linear)	r <sup>2</sup>	g <sub>1</sub> (nls)	RMSE
C3								
		Dicot	<i>Flaveria pringlei</i>	200	2.35	0.91	3.95	0.0290
				380	1.69	0.86	2.54	0.0255
				550	1.40	0.87	1.91	0.0178
				800	1.14	0.86	1.33	0.0125
		Monocot	<i>Panicum bisulcatum</i>	200	2.27	0.82	3.82	0.0363
				380	1.62	0.85	2.39	0.0217
				550	1.41	0.88	1.91	0.0142
				800	1.29	0.89	1.65	0.0105
		Monocot	<i>Steinchisma laxa</i>	200	1.81	0.88	2.81	0.0131
				380	1.32	0.92	1.70	0.0089
				550	1.12	0.92	1.26	0.0062
				800	1.04	0.92	1.07	0.0045
C4								
	NADP-ME	Dicot	<i>Flaveria bidentis</i>	200	1.48	0.76	2.06	0.0667
				380	1.47	0.58	2.07	0.0633
				550	1.44	0.52	1.99	0.0490
				800	1.59	0.41	2.35	0.0491
	NAD-ME	Monocot	<i>Panicum coloratum</i>	200	0.98	0.92	0.95	0.0188
				380	0.97	0.64	0.93	0.0287
				550	0.91	0.59	0.80	0.0181
				800	0.93	0.39	0.84	0.0196
	NADP-ME	Monocot	<i>Panicum maximum</i>	200	1.11	0.89	1.25	0.0375
				380	0.92	0.90	0.82	0.0175
				550	0.84	0.92	0.65	0.0096
				800	0.81	0.90	0.56	0.0064
	PCK	Monocot	<i>Paspalum dilatatum</i>	200	1.33	0.87	1.73	0.0287
				380	1.09	0.81	1.19	0.0157
				550	1.00	0.80	1.01	0.0126
				800	1.02	0.69	1.05	0.0135

### 3.3.4 Unified model fit and calculation of lambda

Figure 3.7 shows the g<sub>1</sub> term as derived from Medlyn et al. (2011) for different species by plotting g<sub>s</sub> as a function of the combination of terms A/Ca√D. Slope or g<sub>1</sub> was comparatively higher for the C3 species (Figure 3.7 a-c) as compared to C4 species (Figure 3.7 d-g) (Table 3.4). Both C3 and C4 species showed a linear relationship between g<sub>s</sub> and the term A/Ca√D. g<sub>s</sub> of C4 species seemed to optimise similar to C3 species but the numerical solution of the stomatal model did not provide a perfect fit of the data (compared to Figure 3.2).

A comparison of lambda (λ) and g<sub>1</sub> values between C3 and C4 species is shown in Table 3.5. Values were calculated by considering two A-Ci curves assumptions as described in methods. For *Flaveria* species, since the A-Ci curves at higher D (2.5 kPa) were considerably low, these values were not included in calculating the λ and g<sub>1</sub> values. λ values

were slightly higher in *Panicum species* (giving a very low  $g_1$ ) but was reduced in *Flaveria* (giving a not so low  $g_1$ ). The overall comparison (weighted t-test) of  $\lambda$  shows that marginal cost of accumulating CO<sub>2</sub> has not changed between C3 and C4 species (Table 3.5).  $g_1$  values however, are significantly lower in C4 species as compared to C3 species.

**Table 3-5:** A comparison of lambda ( $\lambda$ ) and  $g_1$  values between C3 and C4 species. For C3 species  $\lambda$  was calculated from the optimal stomatal behaviour at RuBP-regeneration limited reaction of photosynthesis ( $J_{max}$ ). For C4 species, two methods were used (i) a numerical fit (num) to the von Caemmerer (2000) photosynthesis model and (ii) a rectangular hyperbola function (hypb) to calculate optimal stomatal conductance.  $g_1$  was calculated from non-linear least squared fit of the data. Weighted means were calculated from RMSE and were compared between photosynthetic types. \* denotes p-value<0.05.

C3 Species					C4 Species						
Species name	$\lambda$	RMSE	$g_1$	RMSE	Species name	$\lambda$	RMSE	$\lambda$	RMSE	$g_1$	RMSE
						(num)		(hypb)			
<i>Flaveria pringlei</i>	0.91	0.064	2.63	0.055	<i>Flaveria bidentis</i>	0.17	0.115	0.22	0.061	1.69	0.063
<i>Panicum bisulcatum</i>	0.97	0.045	2.56	0.040	<i>Panicum coloratum</i>	1.38	0.035	1.55	0.023	0.80	0.021
<i>Steinchisma laxa</i>	1.45	0.023	1.96	0.019	<i>Panicum maximum</i>	1.70	0.055	1.99	0.035	0.81	0.025
					<i>Paspalum dilatatum</i>	0.75	0.034	0.92	0.037	1.28	0.028
Mean	1.03		2.49		Mean	0.78		0.98		1.31*	

### 3.4 Discussion

This study evaluated the stomatal responses of different C3 and C4 congener species to changes in  $C_a$  and D, focusing on two key questions: (a) do C4 plants show optimal stomatal behaviour; and (b) how can we best model  $g_s$  of C4 plants. These questions are addressed as follows in the light of the results of the study.

#### *Do C4 plants show optimal stomatal behaviour?*

It was found in the study that C4 plants do follow optimal behaviour and they optimise their  $g_s$  to changing environmental conditions. The unified model by Medlyn et al. (2011) was used to predict optimal behaviour for C4 species, and fitted well with the measured values. The slope  $g_1$  acquired by fitting the unified model gives an insight of how the marginal cost of water ( $\lambda$ ) differs among species. Values of  $g_1$  were found to be lower in C4 species than C3 species (Table 3.5). However, lower  $g_1$  values does not imply a lower  $\lambda$  for the C4 plants, since  $\lambda$  values were found to be similar between C4 and C3 species. From inspection of table 3.5, it appears that C4 plants with the same  $\lambda$  as C3 plants should have approximately half the  $g_1$  values of the C3 plants. The  $g_1$  values are reduced in C4 species compared to C3 species

even if  $\lambda$  remains unchanged, because of the changed nature of the A-C<sub>i</sub> response. In C4 species, a plateau of A was observed, showing saturation of A with increasing C<sub>i</sub>, however for C3 species A was unsaturated even at high C<sub>i</sub>. In C4 species, *F. bidentis* and *P. dilatatum*, the plateau was very prominent, which gave high  $g_1$  values compared to *P. coloratum* and *P. maximum*.

Comparing the C3 and C4 congeners, the marginal carbon cost of water ( $\lambda$ ) was slightly greater in C4 *Panicum* (giving a very low  $g_1$ ) but was lower in C4 *Flaveria* (giving a not so low  $g_1$ ). Since carbon is relatively more available for C4 species, the marginal carbon cost of water might be predicted to be higher in C4 species. In C3 species, *F. pringlei* and *P. bisulcatum* had lower lambda values as compared to *S. laxa*. *S. laxa*, which is considered a relative of the C4 species of *Steinchisma* (Poaceae family) and *Heliotropium* (Boraginaceae family) (Sage et al. 2013), has lambda values close to C4 species of *P. coloratum*, and *P. maximum*. Also the sub-photosynthetic pathway to which these C4 species belong, i.e., NAD-ME and PCK type, are considered to have growth response at high CO<sub>2</sub> more similar to C3 plants (LeCain and Morgan 1998). The A-C<sub>i</sub> response curves revealed that photosynthesis of not all of the C4 species was saturated at present ambient C<sub>a</sub>. A low initial slope and a plateau were seen in *F. bidentis* (NADP-ME dicot) and *P. dilatatum* (NADP-ME monocot) while A was not saturated for *P. coloratum* (NAD-ME) and *P. Maximum* (PCK). Although, close association of photosynthetic types are to be found in same order of families, however, what should be noted is that the  $g_1$  values of C4 species remained nearly half of the  $g_1$  values of C3 species.

The different sub-photosynthetic pathway types in C4 species have been known to respond differently to atmospheric C<sub>a</sub> (LeCain and Morgan 1998, Ghannoum et al. 2000). The unsaturated photosynthetic responses of these C4 species indicate the presence of leakiness of bundle sheath (Hattersley 1982, Ziska et al. 1999) which is defined as the rate of CO<sub>2</sub> leakage divided by the rate of PEP carboxylation (Farquhar 1983). Greater leakiness in a species implies greater photosynthesis and growth responses to eC<sub>a</sub>. Hatch et al. (1995) calculated leakiness by <sup>14</sup>CO<sub>2</sub> pulse chase labelling and found it to be highest in NADP-ME dicots species, followed by NADP-ME monocots, with NAD-ME and PCK type species separated only by marginal differences. In this study the highest leakage or bundle sheath conductance  $g_{bs}$  was estimated for PCK type *P. maximum*, which did not show saturated response to higher C<sub>a</sub>.  $V_{cmax}$  and  $V_{pmax}$  values were also found higher for *P. maximum* compared to rest of the species (Table 3.2). Apart from *P. maximum* all C4 species showed similar  $g_{bs}$  values. Thus, the study did not find any link of C4 leakiness associated with plant photosynthesis responses

to  $eC_a$ . It has also been found in previous reports where different responses from different C4 sub-pathway types were noted (Henderson et al. 1992, Hatch et al. 1995, Ghannoum et al. 1997, LeCain and Morgan 1998, Ziska et al. 1999). Moreover, *Flaveria* species displayed photosynthetic acclimation to growth at high  $D$  of 2.5kPa. This is in consistent to previous works on some C3 and C4 species (Morgan et al. 1994, Read and Morgan 1996). The other grasses however, showed no photosynthetic acclimation.

Stomata of C4 plants were more sensitive to VPD than those of C3 plants. High  $D$  reduced stomatal conductance by about 35% in C3 plants and by 59% in C4 plants, relative to that at  $D$  of 1 kPa (Figure 3.6c). However, the stomatal conductance of C4 plants did not show stronger sensitivity to increasing  $C_a$  than C3 plants. Similar results from Morison & Gifford (1983) and Mehrali et al. (2003) support this conclusion.

The present study was conducted with C3 and C4 plants grown at ambient  $C_a$  and exposed to  $eC_a$  for short periods. Thus, we are characterising the short-term  $eC_a$  response. However, we do not expect long-term responses to  $eC_a$  to be different, based on results from C3 species. Many C3 species grown at  $eC_a$  have been tested against the model and short-term and long-term responses seem to be similar (e.g. De Kauwe et al. 2013). However, reductions in whole plant transpiration under  $eC_a$  can increase soil moisture availability, which indirectly affects stomatal conductance (Grunzweig and Korner 2001, Morgan et al. 2001, Polley et al. 2002). This longer-term feedback effect raises the possibility that  $CO_2$  can indirectly affect stomatal functioning in species via a feedback on soil moisture availability.

#### *How can we model $g_s$ of C4 plants generally?*

Medlyn et al. (2011) used the optimization theory and resolved it with the empirical models based on stomatal conductance measurements. They, however, used the C3 photosynthesis model and assumed that the plants behave as to be optimizing for RuBP regeneration limited reaction. For C4 photosynthesis model, the numerical solution of the stomatal model did not provide a good fit to the data (Figure 3.2b) indicating that the behaviour is not perfectly optimised for the C4 species. However, the unified model by Medlyn et al. (2011) seemed to capture the C4 responses well and predicted the water strategies adopted by the C4 plants by predicting  $\lambda$  values. The unified model offers a source of quantifying  $\lambda$ , by fitting Equation (3) to stomatal conductance measurements and using the fitted parameter  $g_1$  as a proxy for  $\lambda$ . The present study provides  $\lambda$  values for both C3 and C4 species which are found to be similar.



In another approach taken by Way et al. (2014) and Manzoni et al. (2011)  $\lambda$  values were calculated using different equations. To derive a relationship between optimal  $g_s$  and  $A$ , they use a linearised  $A-C_i$  curve. This is quite different from the approach taken in this study, where a rectangular hyperbola (or the full von Caemmerer model) to represent the  $A-C_i$  curve were used. Medlyn et al. (2013) showed that the different approaches lead to different estimates of  $\lambda$  for C3 species. In that paper it was shown that the optimal stomatal behaviour derived by Manzoni et al. (2011) only fits  $CO_2$  responses if  $\lambda$  is assumed proportional to  $C_a$ , whereas the Medlyn et al. (2011) approach fits  $CO_2$  responses with  $\lambda$  constant.

The equation used by Way et al. (2014) to estimate  $\lambda$  was:

$$A_{net} = g_s \sqrt{aD\lambda(C_a - \Gamma^*/\eta)} \quad (11)$$

where the difference between C3 and C4 plants is eta ( $\eta$ ): for C3 plants it is 1, for C4 plants it is around 15 (their Figure 1 b).  $a$  is the ratio of the molecular diffusivities of  $CO_2$  to water vapour ( $=1.6$ ). If  $\eta$  is changed in the above equation from 1 to 15, it will hardly change the slope between  $g_s$  and  $A_{net}$  (for example if  $C_a = 380$ ,  $\Gamma^* = 40$ , the slope changes by 8%). This implies that according to these equations, the optimal  $A$  vs.  $g_s$  relationship for C4 plants is hardly different from that for C3 plants. The present study gives a quite different conclusion because Medlyn et al. (2011) model predict that the slope of the relationship ( $g_1$ ) should halve between C3 and C4 plants with the same  $\lambda$ . So the conclusion drawn depends on what assumption is taken about the shape of the  $A-C_i$  curve, and whether or not it is linearised.

The low  $g_1$  values in C4 species, indicate a distinctive relationship conferred by the higher photosynthetic efficiency relative to C3. With high photosynthetic efficiency, C4 species have optimised their photosynthetic rates but maintain a similar stomatal behaviour as for C3 species thus achieving higher water use efficiency at leaf-level. To adapt themselves at warm temperatures and low  $CO_2$  levels C4 species have managed to concentrate  $CO_2$  more efficiently. It is an important functional innovation in plants of C4 pathway, where it occurs in c. 18 lineages (Kellogg 1999, Sage 2004, Christin et al. 2008, Christin et al. 2009).

There are a number of physical characteristics associated with the evolution of C4 type, including the two-celled photorespiratory concentration mechanism known as C2 photosynthesis (commonly observed in C3–C4 intermediate species) (Muhaidat et al. 2011, Sage et al., 2012), enlarged bundle sheath cells, increased vein density (Sage et al. 2013) and differences in leaf hydraulic conductances to that of C3 (Griffith et al. 2013). Physical differences in stomatal pattern also evolved; lower stomatal conductances are achieved by smaller stomatal pore size and lower densities in dry environments (Taylor et al. 2012).

Higher vein density enables C4 to adapt for water deficit environment which increases hydraulic flux thus maintaining favourable leaf water status (Scoffoni et al., 2011). Higher hydraulic conductance have been hypothesised to be related to low sensitivities of stomates allowing them to remain open during drought, and photosynthesis to continue, but only if C4 species have similar or higher hydraulic conductance than C3 species, and therefore a high hydraulic supply relative to demand (Osbourne and Sack. 2012). Results of the present study essentially indicate the marginal carbon cost of water ( $\lambda$ ) remains the same between C4 and C3 species, but that C4 plants follow the optimal stomatal behaviour, which is to reduce  $g_l$  whilst maintaining higher photosynthetic rates.

### *Conclusions for modelling:*

C4 photosynthesis represents a biochemical and morphological modification of C3 photosynthesis which reduces Rubisco oxygenase activity and thereby increase photosynthetic rate. Since C4 plants have different photosynthetic responses to CO<sub>2</sub>, different stomatal response is expected from them. Coupled photosynthesis and stomatal conductance model by Collatz et al. (1992) gives only the empirical solution whereas the unified optimal stomatal model by Medlyn et al. (2011) has the added advantage of predicting how water use strategy is adapted by C4 species. In the present study, the unified stomatal model predicted that marginal carbon cost of water ‘ $\lambda$ ’ has not changed between C3 and C4 species, however the  $g_l$  values were about half of the values observed for C3 species. This was because of the changed photosynthetic curve with intercellular CO<sub>2</sub> concentrations. Thus, the full numerical solution of the optimal stomatal behaviour model does not accurately predict  $g_s$  responses of C4 species, but the unified model seemed to capture the responses well and accurately predict the C4 plant-water strategies. The unified model is recommended to predict C4 responses to environmental conditions and has extensive potential to act as a framework for interpreting stomatal behaviour across C4 species.

### **References**

- Arneth, A., J. Lloyd, H. Santruckova, M. Bird, S. Grigoryev, Y. N. Kalaschnikov, G. Gleixner, and E. D. Schulze. 2002. Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric CO<sub>2</sub> concentration. *Global Biogeochemical Cycles* 16.
- Ball, J. T. 1988. Thesis: An analysis of stomatal conductance. Submitted to Stanford University, USA.
- Ball, J. T., I. Woodrow, and J. Berry. 1987. A Model Predicting Stomatal Conductance and its Contribution to the Control of Photosynthesis under Different Environmental Conditions. Pages 221-224 in J. Biggins, editor. *Progress in Photosynthesis Research*. Springer Netherlands.

- Barton, C. V. M., R. A. Duursma, B. E. Medlyn, D. S. Ellsworth, D. Eamus, D. T. Tissue, M. A. Adams, J. Conroy, K. Y. Crous, M. Liberloo, M. Low, S. Linder, and R. E. McMurtrie. 2012. Effects of elevated atmospheric  $[CO_2]$  on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. *Global Change Biology* 18:585-595.
- Bauwe, H. 1986. An Efficient Method for the Determination of  $K_m$  Values for  $HCO_3^-$  of Phosphoenolpyruvate Carboxylase. *Planta* 169:356-360.
- Björkman, O., M. D. Hatch, C. B. Osmond, and R. O. Slatyer. 1970. Comparative photosynthetic  $CO_2$  exchange in higher plants. Pages 18-32 in M. D. Hatch, C. B. Osmond, and R. O. Slatyer, editors. *Photosynthesis and photorespiration*. Wiley Interscience, New York, NY, USA.
- Cao, L., G. Bala, K. Caldeira, R. Nemani, and G. Ban-Weiss. 2010. Importance of carbon dioxide physiological forcing to future climate change. *Proceedings of the National Academy of Sciences of the United States of America* 107:9513-9518.
- Christin, P.-A., N. Salamin, E. A. Kellogg, A. Vicentini, and G. Besnard. 2009. Integrating Phylogeny into Studies of C4 Variation in the Grasses. *Plant Physiology* 149:82-87.
- Christin, P. A., G. Besnard, E. Samaritani, M. R. Duvall, T. R. Hodkinson, V. Savolainen, and N. Salamin. 2008. Oligocene  $CO_2$  decline promoted C-4 photosynthesis in grasses. *Current Biology* 18:37-43.
- Collatz, G. J., J. T. Ball, C. Grivet, and J. A. Berry. 1991. Physiological and Environmental-Regulation of Stomatal Conductance, Photosynthesis and Transpiration - a Model That Includes a Laminar Boundary-Layer. *Agricultural and Forest Meteorology* 54:107-136.
- Collatz, G. J., M. Ribas-Carbo, and J. A. Berry. 1992. Coupled Photosynthesis-Stomatal Conductance Model for Leaves of C4 Plants. *Australian Journal of Plant Physiology* 19:519-538.
- Cowan, I. R. 1982. Regulation of Water Use in Relation to Carbon Gain in Higher Plants. Pages 589-613 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. *Physiological Plant Ecology II*. Springer Berlin Heidelberg.
- Cowan, I. R. and G. D. Farquhar. 1977. Stomatal function in relation to leaf metabolism and environment. *Symp Soc Exp Biol* 31:471-505.
- De Kauwe, M. G., B. E. Medlyn, S. Zaehle, A. P. Walker, M. C. Dietze, T. Hickler, A. K. Jain, Y. Q. Luo, W. J. Parton, I. C. Prentice, B. Smith, P. E. Thornton, S. S. Wang, Y. P. Wang, D. Warland, E. S. Weng, K. Y. Crous, D. S. Ellsworth, P. J. Hanson, H. Seok Kim, J. M. Warren, R. Oren, and R. J. Norby. 2013. Forest water use and water use efficiency at elevated  $CO_2$ : a model-data intercomparison at two contrasting temperate forest FACE sites. *Global Change Biology* 19:1759-1779.
- Duursma, R. A. 2012. GasExchangeR: Leaf gas exchange models. R package version 1.4.5.
- Duursma, R. A. 2014. plantecophys: Leaf gas exchange models. R package version 0.3.
- Edwards, E. J., C. P. Osborne, C. A. E. Strömberg, S. A. Smith, and C. G. Consortium. 2010. The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science* 328:587-591.
- Edwards, E. J. and S. A. Smith. 2010. Phylogenetic analyses reveal the shady history of C-4 grasses. *Proceedings of the National Academy of Sciences of the United States of America* 107:2532-2537.
- Ehleringer, J. R., T. E. Cerling, B. R. Helliker. 1997. C4 photosynthesis, atmospheric  $CO_2$  and climate. *Oecologia* 112(3): 285-299.
- Farquhar, G. D. 1983. On the Nature of Carbon Isotope Discrimination in C-4 Species. *Australian Journal of Plant Physiology* 10:205-226.
- Farquhar, G. D., S. Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic  $CO_2$  assimilation in leaves of C3 species. *Planta* 149:78-90.

- Farquhar, G. D. and S. von Caemmerer. 1982. Modelling of Photosynthetic Response to Environmental Conditions. Pages 549-587 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. Physiological Plant Ecology II. Springer Berlin Heidelberg.
- Gedney, N., P. M. Cox, R. A. Betts, O. Boucher, C. Huntingford, and P. A. Stott. 2006. Detection of a direct carbon dioxide effect in continental river runoff records. *Nature* 439:835-838.
- Ghannoum, O. 2009. C-4 photosynthesis and water stress. *Annals of Botany* 103:635-644.
- Ghannoum, O., S. V. Caemmerer, L. H. Ziska, and J. P. Conroy. 2000. The growth response of C4 plants to rising atmospheric CO<sub>2</sub> partial pressure: a reassessment. *Plant, Cell & Environment* 23:931-942.
- Ghannoum, O., S. vonCaemmerer, E. W. R. Barlow, and J. P. Conroy. 1997. The effect of CO<sub>2</sub> enrichment and irradiance on the growth, morphology and gas exchange of a C-3 (*Panicum laxum*) and a C-4 (*Panicum antidotale*) grass (vol 24, pg 227, 1997). *Australian Journal of Plant Physiology* 24:U2-U2.
- Griffiths, H., G. Weller, L. F. M. Toy, and R. J. Dennis. 2013. You're so vein: bundle sheath physiology, phylogeny and evolution in C3 and C4 plants. *Plant, Cell & Environment* 36:249-261.
- Grunzweig, J. M. and C. Korner. 2001. Growth, water and nitrogen relations in grassland model ecosystems of the semi-arid Negev of Israel exposed to elevated CO<sub>2</sub>. *Oecologia* 128:251-262.
- Hari, P., A. Makela, E. Korpilahti, and M. Holmberg. 1986. Optimal control of gas exchange. *Tree Physiology* 2:169-175.
- Hatch, M. D. 1987. C-4 Photosynthesis - a Unique Blend of Modified Biochemistry, Anatomy and Ultrastructure. *Biochimica Et Biophysica Acta* 895:81-106.
- Hatch, M. D., A. Agostino, and C. L. D. Jenkins. 1995. Measurement of the Leakage of CO<sub>2</sub> from Bundle-Sheath Cells of Leaves during C-4 Photosynthesis. *Plant Physiology* 108:173-181.
- Hattersley, P. W. 1982. Delta-C-13 Values of C-4 Types in Grasses. *Australian Journal of Plant Physiology* 9:139-154.
- Henderson, S. A., S. Voncaemmerer, and G. D. Farquhar. 1992. Short-Term Measurements of Carbon Isotope Discrimination in Several C4 Species. *Australian Journal of Plant Physiology* 19:263-285.
- Herault, A., Y. S. Lin, A. Bourne, B. E. Medlyn, and D. S. Ellsworth. 2013. Optimal stomatal conductance in relation to photosynthesis in climatically contrasting Eucalyptus species under drought. *Plant Cell and Environment* 36:262-274.
- Jensen, C. R., H. Svendsen, M. N. Andersen, and R. Losch. 1992. Use of the Root Contact Concept, an Empirical Leaf Conductance Model and Pressure-Volume Curves in Simulating Crop Water Relations. *Second Congress of the European Society for Agronomy*:180-181.
- Katul, G., S. Manzoni, S. Palmroth, and R. Oren. 2010. A stomatal optimization theory to describe the effects of atmospheric CO<sub>2</sub> on leaf photosynthesis and transpiration. *Annals of Botany* 105:431-442.
- Kellogg, E. A. 1999. 12 - Phylogenetic Aspects of the Evolution of C4 Photosynthesis. Pages 411-444 in R. F. Sage and R. K. Monson, editors. *C4 Plant Biology*. Academic Press, San Diego.
- Kürrats, O., P. J. Lea, V. R. Franceschi, and G. E. Edwards. 2003. Bundle sheath diffusive resistance to CO<sub>2</sub> and effectiveness of C-4 photosyntheses and refixation of photorepired CO<sub>2</sub> in a C-4 cycle mutant and wild-type *Amaranthus edulis* (vol 130, pg 964, 2002). *Plant Physiology* 132:400-400.
- Larcher, W. 1995. *Physiological plant ecology*. Springer-Verlag., Berlin.
- Laik, A. and G. E. Edwards 1997. "Post-illumination CO<sub>2</sub> Exchange and Light-induced CO<sub>2</sub> Bursts during C4 Photosynthesis." *Functional Plant Biology* 24(4): 517-528.

- LeCain, D. R. and J. A. Morgan. 1998. Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C-4 grasses grown in elevated CO<sub>2</sub>. *Physiologia Plantarum* 102:297-306.
- Leuning, R. 1990a. Modeling Stomatal Behavior and Photosynthesis of *Eucalyptus-Grandis*. *Australian Journal of Plant Physiology* 17:159-175.
- Leuning, R. 1990b. Modelling Stomatal Behaviour and Photosynthesis of *Eucalyptus grandis*. *Functional Plant Biology* 17:159-175.
- Leuning, R., F. M. Kelliher, D. G. G. Depury, and E. D. Schulze. 1995. Leaf Nitrogen, Photosynthesis, Conductance and Transpiration - Scaling from Leaves to Canopies. *Plant Cell and Environment* 18:1183-1200.
- Lloyd, J. 1991. Modeling Stomatal Responses to Environment in *Macadamia-Integrifolia*. *Australian Journal of Plant Physiology* 18:649-660.
- Lloyd, J., O. Shibistova, D. Zolotoukhine, O. Kolle, A. Arneth, C. Wirth, J. M. Styles, N. M. Tchebakova, and E. D. Schulze. 2002. Seasonal and annual variations in the photosynthetic productivity and carbon balance of a central Siberian pine forest. *Tellus Series B-Chemical and Physical Meteorology* 54:590-610.
- Long, S. P. 1999. 7 - Environmental Responses. Pages 215-249 in R. F. Sage and R. K. Monson, editors. *C4 Plant Biology*. Academic Press, San Diego.
- Maherali, H., H. B. Johnson, and R. B. Jackson. 2003. Stomatal sensitivity to vapour pressure difference over a subambient to elevated CO<sub>2</sub> gradient in a C-3/C-4 grassland. *Plant Cell and Environment* 26:1297-1306.
- Manzoni, S., G. Vico, G. Katul, P. A. Fay, W. Polley, S. Palmroth, and A. Porporato. 2011. Optimizing stomatal conductance for maximum carbon gain under water stress: a meta-analysis across plant functional types and climates. *Functional Ecology* 25:456-467.
- McMurtrie, R. E., R. Leuning, W. A. Thompson, and A. M. Wheeler. 1992. A Model of Canopy Photosynthesis and Water-Use Incorporating a Mechanistic Formulation of Leaf CO<sub>2</sub> Exchange. *Forest Ecology and Management* 52:261-278.
- Medlyn, B. E., R. A. Duursma, M. G. De Kauwe, and I. C. Prentice. 2013. The optimal stomatal response to atmospheric CO<sub>2</sub> concentration: Alternative solutions, alternative interpretations. *Agricultural and Forest Meteorology* 182:200-203.
- Medlyn, B. E., R. A. Duursma, D. Eamus, D. S. Ellsworth, I. C. Prentice, C. V. M. Barton, K. Y. Crous, P. de Angelis, M. Freeman, and L. Wingate. 2011. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17:2134-2144.
- Morgan, J. A., H. W. Hunt, C. A. Monz, and D. R. LeCain. 1994. Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C3) and *Bouteloua gracilis* (C4). *Plant, Cell & Environment* 17:1023-1033.
- Morgan, J. A., D. R. LeCain, A. R. Mosier, and D. G. Milchunas. 2001. Elevated CO<sub>2</sub> enhances water relations and productivity and affects gas exchange in C-3 and C-4 grasses of the Colorado shortgrass steppe. *Global Change Biology* 7:451-466.
- Morison, J. I. L. and R. M. Gifford. 1983. Stomatal Sensitivity to Carbon-Dioxide and Humidity - a Comparison of 2 C-3 and 2 C-4 Grass Species. *Plant Physiology* 71:789-796.
- Muhaidat, R., T. L. Sage, M. W. Frohlich, N. G. Dengler, and R. F. Sage. 2011. Characterization of C3-C4 intermediate species in the genus *Heliotropium* L. (Boraginaceae): anatomy, ultrastructure and enzyme activity. *Plant, Cell & Environment* 34:1723-1736.
- Norman, J. M. and W. Polley. 1989. Canopy Photosynthesis. Pages 227-241 *Photosynthesis*. Alan Liss, New York.

- Osborne, C. P. and L. Sack. 2012. Evolution of C4 plants: a new hypothesis for an interaction of CO<sub>2</sub> and water relations mediated by plant hydraulics. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 367:583-600.
- Polley, H. W., H. B. Johnson, and J. D. Derner. 2002. Soil- and plant-water dynamics in a C3/C4 grassland exposed to a subambient to superambient CO<sub>2</sub> gradient. *Global Change Biology* 8:1118-1129.
- R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Read, J. J. and J. A. Morgan. 1996. Growth and partitioning in *Paspopyrum smithii* (C3) and *Bouteloua gracilis* (C4) as influenced by carbon dioxide and temperature. *Annals of Botany* 77:487-496.
- Sage, R. and D. Kubien. 2003. Quo vadis C4? An ecophysiological perspective on global change and the future of C4 plants. *Photosynthesis Research* 77:209-225.
- Sage, R. F. 2002. Variation in the *k<sub>cat</sub>* of Rubisco in C3 and C4 plants and some implications for photosynthetic performance at high and low temperature. *Journal of Experimental Botany* 53:609-620.
- Sage, R. F. 2004. The evolution of C4 photosynthesis. *New Phytologist* 161:341-370.
- Sage, R. F., T. L. Sage, and F. Kocacinar. 2012. Photorespiration and the Evolution of C4 Photosynthesis. *Annual Review of Plant Biology* 63:19-47.
- Sage, T. L., F. A. Busch, D. C. Johnson, P. C. Friesen, C. R. Stinson, M. Stata, S. Sultmanis, B. A. Rahman, S. Rawsthorne, and R. F. Sage. 2013. Initial Events during the Evolution of C4 Photosynthesis in C3 Species of Flaveria. *Plant Physiology* 163:1266-1276.
- Scoffoni, C., M. Rawls, A. McKown, H. Cochard, and L. Sack. 2011. Decline of Leaf Hydraulic Conductance with Dehydration: Relationship to Leaf Size and Venation Architecture. *Plant Physiology* 156:832-843.
- Sellers, P. J., J. A. Berry, G. J. Collatz, C. B. Field, and F. G. Hall. 1992. Canopy Reflectance, Photosynthesis, and Transpiration .3. A Reanalysis Using Improved Leaf Models and a New Canopy Integration Scheme. *Remote Sensing of Environment* 42:187-216.
- Taylor, S. H., P. J. Franks, S. P. Hulme, E. Spriggs, P. A. Christin, E. J. Edwards, F. I. Woodward, and C. P. Osborne. 2012. Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. *New Phytologist* 193:387-396.
- Taylor, S. H., S. P. Hulme, M. Rees, B. S. Ripley, F. Ian Woodward, and C. P. Osborne. 2010. Ecophysiological traits in C3 and C4 grasses: a phylogenetically controlled screening experiment. *New Phytologist* 185:780-791.
- Thomas, D. S., D. Eamus, and D. Bell. 1999. Optimization theory of stomatal behaviour - I. A critical evaluation of five methods of calculation. *Journal of Experimental Botany* 50:385-392.
- Tilman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science* 314:1598-1600.
- von Caemmerer, S. 2000. Biochemical models of leaf photosynthesis / S. von Caemmerer. CSIRO Publishing, Collingwood, Vic.
- von Caemmerer, S. and R. T. Furbank. 1999. Modeling C4 Photosynthesis. Pages 173-211.
- Wand, S. J. E., G. F. Midgley, M. H. Jones, and P. S. Curtis. 1999. Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO<sub>2</sub> concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* 5:723-741.

- Watling, J. R. and M. C. Press. 1997. How is the relationship between the C-4 cereal *Sorghum bicolor* and the C-3 root hemi-parasites *Striga hermonthica* and *Striga asiatica* affected by elevated CO<sub>2</sub>? Plant Cell and Environment 20:1292-1300.
- Way, D. A., G. G. Katul, S. Manzoni, and G. Vico. 2014. Increasing water use efficiency along the C3 to C4 evolutionary pathway: a stomatal optimization perspective. Journal of Experimental Botany 65:3683-3693.
- Wong, S. C. 1979. Elevated Atmospheric Partial Pressure of CO<sub>2</sub> and Plant Growth. I. Interactions of Nitrogen Nutrition and Photosynthetic Capacity in C4 and C4 Plants. Oecologia 44:68-74.
- Zeiger, E. 1983. The Biology of Stomatal Guard-Cells. Annual Review of Plant Physiology and Plant Molecular Biology 34:441-475.
- Zeiger, E., Farquhar, G. D., & Cowan, I. R. . 1987. Stomatal Function. Stanford University Press, Stanford, California.
- Zhang, J. X. and M. B. Kirkham. 1995. Water Relations of Water-Stressed, Split-Root C4 (*Sorghum-Bicolor* Poaceae) and C3 (*Helianthus-Annuus* Asteraceae) Plants. American Journal of Botany 82:1220-1229.
- Ziska, L. H. and J. A. Bunce. 1997. Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C4 crops and weeds. Photosynthesis Research 54:199-208.
- Ziska, L. H., R. C. Sicher, and J. A. Bunce. 1999. The impact of elevated carbon dioxide on the growth and gas exchange of three C4 species differing in CO<sub>2</sub> leak rates. Physiologia Plantarum 105:74-80.

## CHAPTER 4

### Is plant water use efficiency proportional to atmospheric CO<sub>2</sub>?

**Summary** Elevated CO<sub>2</sub> (eC<sub>a</sub>) increases net photosynthetic rates in plants and reduces water loss through stomatal conductance (g<sub>s</sub>). Hence, plant water use efficiency, taken as a ratio of photosynthesis to transpiration, is often increased with increasing CO<sub>2</sub> concentration (C<sub>a</sub>). Models predict that the increase in plant water use efficiency should be proportional to increase in C<sub>a</sub> and should be uniform across vegetation types. The aims of this study were to firstly, determine whether the experimental data from the literature support this prediction i.e. this proportionality is present at both leaf and whole plant scale levels, and secondly, to test for differences among plant functional types. By using meta-analysis techniques, the relative plant responses to eC<sub>a</sub> were determined. The results revealed that with 50% increase in C<sub>a</sub>, overall, species showed 56%, 53% and 33% increases in intrinsic water use efficiency (A/g<sub>s</sub> = iWUE), instantaneous transpiration efficiency (A/E = iTE) and whole plant water use efficiency (Biomass/ E = WUE) respectively. A 100% increase in C<sub>a</sub> resulted in 76%, 76% and 48% increases in iWUE, iTE and WUE respectively. Using a meta-regression approach, where each data point was normalised by its respective C<sub>a</sub>, we found that where there was a 1:1 increase in iWUE, iTE and WUE with CO<sub>2</sub>, iWUE, iTE and WUE increased in an overall proportion of 0.94, 0.92 and 0.79 respectively among studies. Results from both methods indicated that at leaf level, iWUE and iTE increase in proportion to the increase in C<sub>a</sub> for C3 herbs, C4 herbs and trees, whereas at whole plant level, WUE is on average less than proportional to C<sub>a</sub>.

#### 4.1 Introduction

The atmospheric carbon dioxide concentration (C<sub>a</sub>) has increased from the pre-industrial value of approximately 280 μmol mol<sup>-1</sup> to the current value near to 400 μmol mol<sup>-1</sup> (IPCC 2007, NOAA 2011). The terrestrial biosphere responds to rising C<sub>a</sub> chiefly through the response of plants (Hughes 2000, Korner 2000, Poorter and Navas 2003, Nowak et al. 2004, Norby et al. 2005, Holmes et al. 2006, Taneva et al. 2006, Zak et al. 2011, Talhelm et al. 2012, Zak et al. 2012). Photosynthesis (Long and Drake 1992) and transpiration (Heath 1948) have long been known to be sensitive to increases in C<sub>a</sub>, leading to stimulation of plant growth and biomass production (Drake et al. 1997, Centritto et al. 2002, Li et al. 2002, Ainsworth and Long 2005, King et al. 2005). Because photosynthesis is increased and



transpiration is decreased, the benefit of  $eC_a$  for plant growth can be expressed as an increase in water-use efficiency (WUE), which is the ratio of net photosynthesis ( $A$ ) to water loss through transpiration ( $E$ ). The positive effect of  $eC_a$  on the water-use efficiency of plants has been documented in single-species studies (Koike et al. 1996, Wayne et al. 1998, De Luis et al. 1999, Greenep et al. 2003, Vu 2005, Cao et al. 2007, Wertin et al. 2010) and multi-species studies (Tschaplinski et al. 1995, Ball et al. 1997, Kubiske and Pregitzer 1997, Saxe et al. 1998, Tjoelker et al. 1998, Wullschlegel et al. 2002, Nowak et al. 2004, Ghannoum et al. 2010, Hovenden and Williams 2010, Zheng et al. 2010, Cernusak et al. 2011). Although there is strong evidence that WUE responds strongly to  $eC_a$ , both at the leaf and whole plant scale (Morison 1993, Overdieck and Forstreuter 1994, Picon et al. 1996, Morgan et al. 2001), there has been relatively little focus on “how much” it changes by, and in particular, whether the experimental data support predicted increases in WUE by vegetation models.

A key process in determining water use efficiency is stomatal conductance, which plays an essential role in the regulation of both water losses by transpiration and  $CO_2$  uptake for photosynthesis and plant growth (Brakke and Allen 1995, Saxe et al. 1998). Stomatal conductance is among the processes that have been most extensively modelled during the last decades (Damour et al. 2010). Stomatal conductance models are typically parameterized using field measurements of leaf-level gas exchange. One widely-used approach for modelling leaf-level stomatal conductance (Ball et al. 1987, Leuning 1995, Medlyn et al. 2011) has the form;

$$g_s = g_0 + m \frac{A}{C_a f(D)} \quad (1)$$

Where  $g_s$  is the stomatal conductance to water vapour,  $g_0$  is the stomatal conductance at the light compensation point,  $m$  is a fitting parameter representing the slope of the equation,  $A$  is photosynthesis,  $f(D)$  is the function of vapour pressure deficit ( $D$ ) or relative humidity (RH) and  $C_a$  is the molar fraction of  $CO_2$  at the leaf surface. In simple models  $g_0$  is assumed to be zero. If  $D$  is assumed unchanged at  $eC_a$  and  $g_0$  is small, then equation (1) can be rearranged to give

$$\frac{A}{g_s} \propto C_a \quad (2)$$

Equation (2) shows that leaf level intrinsic water-use efficiency ( $A/g_s$ ) is predicted to increase linearly as  $C_a$  increases. In addition, in canopies where plants are “well-coupled” to their surrounding atmospheric conditions, the transpiration rate is largely determined by stomatal conductance. i.e. transpiration ( $E$ ) ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) can be estimated as:

$$E = g_s \frac{D}{p} \quad (3)$$

where  $P$  is the atmospheric pressure (kPa). If  $E$  is assumed to vary in proportion to  $g_s$  following equation (3), then  $A/E$  is also predicted to be proportional to  $C_a$ .

$$\frac{A}{E} \propto C_a \quad (4)$$

If these leaf-level equations could be extrapolated to whole-plant level, and respiratory losses were a constant proportion of photosynthetic uptake (as is often the case, e.g. (Gifford 1995), the effect of  $C_a$  on biomass production would be predicted to be proportional to its effect on plant assimilation rate. In this case, plant biomass production per unit total plant transpiration, or whole plant water use efficiency (WUE), would also be predicted to increase in proportion to  $C_a$ .

However, leaf level predictions (equations 2 & 4) translate directly to the natural ecosystems only when there is minimal negative feedback effect of leaf surface temperature and atmospheric humidity on the sensitivity of transpiration to a change in leaf conductance. The sensitivity of transpiration or evapotranspiration to changes in stomatal aperture has been shown to decrease progressively from an individual stoma, to leaves, whole plants, canopies and extensive vegetated surfaces (Jarvis and McNaughton 1986). Stomatal apertures facilitate the molecular diffusion of water vapour from the sub-stomatal cavity to the outside air. The air immediately next to the stomatal opening near the leaf surface is motionless. This air is often termed as leaf boundary layer. The subsequent layers above boundary layer have turbulent air movements which remove water vapour more efficiently than the molecular diffusion in sub-stomatal cavities. When boundary layer resistance is low, or air around leaf is in high motion, stomata exert maximum control over gas exchange. The leaf is said to be “well-coupled” in this condition. Jarvis and McNaughton (1986) use the term “omega factor” a dimensionless number between 0 and 1, to refer to the degree to which transpiration is coupled or uncoupled from the atmospheric water vapour saturation deficit. When an atmospheric saturation deficit exists at the leaf surface level, transpiration is directly influenced by the vapour gradient and by surface conductance of the foliage, and omega approaches zero as indicated by equation (3). This condition exists when atmospheric mixing is sufficient. However, when mixing is scarce, for example over a surface of low stature canopy, with a dense canopy, or when air movement is quite low, the control placed on the movement of water at the leaf surface by atmospheric demand is low, because the canopy becomes decoupled from the airstream. The stomatal regulation of water vapour movement is reduced as observed by Bange (1953), and transpiration is more directly related to radiation input (Jarvis 1985). Under these conditions, omega approaches 1. Generally, transpiration is

the result of both aerodynamic and radiation-driven processes occurring simultaneously, and the omega factor lies between 0 and 1.

Under changing environmental conditions, transpiration or  $E$  does not change proportionally to changes in  $g_s$ . Therefore, we might expect that the  $C_a$  effect on whole-plant WUE would be less than proportional to  $C_a$ , depending on the strength of the coupling between plant and the atmosphere. Process-based models which show low coupling between vegetation-atmosphere feedback processes, predict much smaller  $C_a$  effect on WUE than a model with high coupling (De Kauwe et al. 2013). However, currently we don't have a good estimate for how much WUE is increased, relative to the increase in  $C_a$ .

Furthermore, the simple models (equation 2 & 4) presented above predict that WUE will increase in proportion to  $eC_a$  for all plant species. Experimental evidence however, suggests that there may be differences in responses among different species types, as there are differences in the  $C_a$  effects on photosynthetic rates or in transpiration rates. One difference is between C3 and C4 plants. Positive growth responses to  $eC_a$  although, have been reported for a both C3 and C4 plants, it is generally smaller in C4 than that observed in C3 species (Kimball et al. 1993, Poorter 1993, Ghannoum et al. 2000, Long et al. 2004).  $CO_2$  enrichment has a larger effect on C3 plants than C4 plants because C3 plants increase their rates of photosynthesis more than C4 plants. Therefore, a larger  $CO_2$  effect on WUE is often predicted for C3 species than C4 species. However, in Chapter 3, I showed that  $CO_2$  affects the ratio  $A/g_s$  similarly in C3 and C4 plants. Thus, there are also reasons to predict that WUE will be similarly increased in C3 and C4 plants.

A second difference among functional types is that it has been shown that stomatal responses to  $C_a$  decrease from herbs and crops to trees (Bryant et al. 1998, Curtis and Wang 1998, Saxe et al. 1998, Pataki et al. 2000). This difference in stomatal response could potentially change the  $CO_2$  effect on WUE. Woody species in particular might differ from more rapidly metabolizing C3 grass and forb species in this regard. Most conifer species show small or non-significant responses of  $g_s$  to  $CO_2$  in the field (Saxe et al. 1998, Medlyn et al. 2001, Ainsworth and Long 2005). Herbaceous species and grasses generally show a large  $CO_2$  response of stomata whereas deciduous trees often show a more moderate  $CO_2$  response (Saxe et al. 1998). The order of  $g_s$  sensitivity to  $CO_2$ -increases across different vegetation groups was suggested by Robinson (1994), Knapp et al. (1996) and Saxe et al. (1998).

In a study by Brodribb et al. (2009), stomatal responses to ambient and elevated  $CO_2$  were compared in a diverse range of higher angiosperms, conifers, ferns and lycophytes. They found that the angiosperms have mechanisms for detecting and responding to increases in  $C_a$

that are absent from earlier diverging lineages. Angiosperm stomata were found to be highly sensitive to both decreased and elevated  $C_a$ , imparting greater capacity to optimize water-use efficiency. The ability to sustain higher stomatal conductance rates in angiosperms is due to higher stomatal densities of smaller stomata than gymnosperms and pteridophytes (Hetherington and Woodward, 2003; Franks et al., 2009) allowing higher diffusible area of stomatal pore relative to the total leaf area (Haworth et al. 2011). Thus, an evolutionary trend is also evident in the stomatal responses of plant groups to  $CO_2$ .

In the light of these studies we expect different stomatal responses from different plant function types to elevated  $CO_2$ . If stomatal responses are in order described by Saxe et al. (1998) and Brodribb et al. (2009) (i.e more in grasses and less in conifers, and more in angiosperm and less in conifers), and photosynthetic responses are similar among functional groups, we would expect the  $CO_2$  effect on WUE to be largest for grasses and smallest for gymnosperms.

Another important aspect in the difference among plant functional types is the difference in the sensitivity of transpiration to stomatal conductance at the plant ecosystem level. Crops and herbs, due to their shorter canopies, show poorer aerodynamic coupling of vegetation to the atmospheric surface layer than tall forests. Tall forest canopies experience a more turbulent and faster air-stream, which results in their leaves being well-coupled to the atmosphere around them. Leaf size, morphology and wind speed are also thought to control boundary layer conductance (Monteith and Unsworth 1990, Nobel 1991, Schuepp 1993). Because of the relationship between leaf size and boundary layer conductance, conifers are assumed to have very large boundary layer conductance as compared to broad-leaf trees (Geller and Smith 1982, Martin et al. 1999). Thus, although, crops show the largest reductions in stomatal conductance at  $eCO_2$  level (Bunce 2004) among any vegetation type, the effect may be offset because of the reduced coupling to the atmosphere. Hence it is also possible that the whole-plant water use efficiency of woody vegetation may respond more strongly to  $eC_a$  than that of herbaceous vegetation, due to the stronger aerodynamic coupling (Eamus and Jarvis 1989).

The different responses among plant functional types and between leaf and canopy scales, suggests that the  $CO_2$  effect on WUE may not correspond well to the model prediction of a proportional change with  $C_a$ . The primary purpose of this review is to determine whether this proportionality (equation 2 & 4) holds at both leaf and plant scales. The second goal of the review is to test for differences among plant functional types. A meta-analysis was

conducted on previous experimental outcomes to test alternative hypotheses for the magnitude of the CO<sub>2</sub> effect on water use efficiency of plants.

Water use efficiency can be defined in several different ways. For clarity, I define three different terms: (1) instantaneous transpiration efficiency (iTE), is defined as leaf level photosynthesis divided by transpiration ( $A/E$ ,  $\mu\text{molCO}_2 \text{ (mmolH}_2\text{O)}^{-1}$ ); (2) intrinsic water use efficiency (iWUE), is defined as leaf-level photosynthesis divided by stomatal conductance ( $A/g_s$ ,  $\text{mmolCO}_2 \text{ (molH}_2\text{O)}^{-1}$ ); and (3) whole-plant water use efficiency (WUE), is defined as biomass increment per unit total transpiration ( $\text{g DM (kg H}_2\text{O)}^{-1}$  or  $\text{g of CO}_2/\text{m}^2/\text{y/mm of H}_2\text{O}$ ). The question is addressed at leaf scale, whether, iTE and iWUE respond in proportion to  $C_a$ ; and whether this increase translates into a proportional change on whole-plant WUE.

In addition, I looked at different plant functional types (PFTs). One hypothesis is that, as models predict,  $A/E$  should be proportional to  $C_a$  in all plant species. The alternative hypothesis that was tested is that PFTs differ, according to their different sensitivity of photosynthesis and  $g_s$ . It was hypothesized that WUE of C4 plants would be less responsive to CO<sub>2</sub> than that of C3 plants; and that WUE among C3 plants would respond in this order: C3-herbs/crops > angiosperm trees > gymnosperm trees. The ultimate goal was to understand how well the existing experimental literature supports model predictions.

## 4.2 Materials and methods

### 4.2.1 Data collection:

Data were collected by searching ISI Web of Science for peer-reviewed journal articles between 1980 and 2014. The search included the terms like, “Elevated CO<sub>2</sub> and water use efficiency of plants”, “CO<sub>2</sub> enrichment and plant water use” or “Elevated CO<sub>2</sub> and transpiration efficiency”. Only articles reporting effect of CO<sub>2</sub> concentration on WUE, iTE and iWUE with standard errors were considered for meta-analysis. If these articles reported response variables associated with WUE, iTE and iWUE such as, photosynthesis, biomass increment, stomatal conductance and transpiration rates, these values were also recorded for the meta-analysis.

### 4.2.2 Data categorization:

Only studies with at least two CO<sub>2</sub> treatment groups, control and  $eC_a$  were included. In each experiment, the lowest or nearest to ambient  $C_a$  was taken as the control. The control

treatment  $C_a$  varied from  $200 \mu\text{mol mol}^{-1}$  to  $450 \mu\text{mol mol}^{-1}$ , whereas the  $eC_a$  treatment varied from  $500 \mu\text{mol mol}^{-1}$  to  $1500 \mu\text{mol mol}^{-1}$ . In the analysis, only the difference between higher and lower values of  $\text{CO}_2$  was taken, it did not depend on how low the  $\text{CO}_2$  values were. If a study reported more than two  $\text{CO}_2$  treatments, the lowest treatment was taken as the control and the two higher treatments were taken as elevated treatments. One study reported four  $\text{CO}_2$  treatments which were paired into two. Different  $\text{CO}_2$  fumigation facilities, such as growth chambers, glasshouses, open-top chambers or Free Air Carbon dioxide Enrichment (FACE) were used.

Studies that reported data for different species were considered as independent whereas any study which had additional drought treatment along with well-watered treatment, only the later study was selected for the analysis. Studies which had nutrient treatments such as low or highly fertilized were considered independent as no detailed description of soil type was given in the experiments. Moreover, different studies used different levels and types of nutrients added which cannot be precisely grouped into high or low nutrient treatments. Studies that had other manipulations, such as temperature, soil type etc., data from these studies were taken as independent.

Whole plant or canopy transpiration was calculated as “water loss” in the studies. “Water loss” or canopy transpiration was calculated as the difference in pot weights after applying water to 100% field capacity. Plants were usually covered with straws or fine stones to minimize water loss from soil surface.

Data were categorized according to WUE, iTE and iWUE values for C3 and C4 plants. These were further categorized into herbaceous, crops, angiosperms and gymnosperms. To extract one single value from studies with repeated measures, a fixed effect method was used to calculate average effect (Lajeunesse 2011). There were few studies on gymnosperms as compared to other plant functional types (see results).

#### 4.2.3 Data analysis:

Meta-analysis was carried out according to the methods described by Lajeunesse (2011) which explains meta-analysis of response ratios for studies with correlated and multi-group designs.

The log response ratio (RR) (Hedges et al. 1999), is used as a common effect size measure for the meta-analysis of ecological research, which quantifies the response in a simple two-group experimental design as:

$$\ln RR = \ln \left( \frac{X_T}{X_C} \right) \quad (5)$$

Here, the response ratio  $\ln RR$  is the natural-log proportional change in the means ( $X$ ) of a treatment (T) and control group (C).

When pooling RR from multiple studies, weights are assigned to each RR which are inversely proportional to their sampling variance:

$$\sigma^2(RR) = \frac{(SD_C)^2}{(N_C X_C)^2} + \frac{(SD_T)^2}{(N_T X_T)^2} \quad (6)$$

where SD and N are the standard deviation and sample size of  $X_T$  and  $X_C$ , respectively (Hedges and Olkin 1985, Hedges et al. 1999). A random-effect model was used to combine studies; this method takes into account between-study variances.

Two approaches were taken to test for the  $CO_2$  effect on plant WUE. The first approach was a standard meta-analysis of the  $CO_2$  response of WUE. As different responses were expected for different  $CO_2$  treatments, the experiments were partitioned into those where the  $CO_2$  increase applied was less than 70% (small  $CO_2$  increment) and those where it was greater than 70% (large  $CO_2$  increment). Most of the studies clumped around 50% and 100%, therefore 70% was taken as a dividing point. Studies which were grouped into small  $CO_2$  increment had an average  $CO_2$  increment of 50% whereas studies which were grouped in large  $CO_2$  increment had an average  $CO_2$  increment of 100%. The ' $CO_2$  increment' was treated as a categorical variable. The response variable, %  $CO_2$  effect or 'effect size', was calculated by taking the antilog of  $\ln RR$ ,  $(RR-1) \times 100$ . A mixed model was used to partition total heterogeneity within and between levels of each categorical variable and tested for significant between-group heterogeneity with respect to  $CO_2$  increase.  $iWUE$ ,  $iTE$  and  $WUE$  were predicted to increase in proportion to  $CO_2$  increment, i.e. 50% for small group and 100% for large group.

To determine whether the increase in water use efficiency was principally due to an increase in carbon uptake or a decrease in water use, it was also examined how photosynthesis, biomass, stomatal conductance and transpiration varied in response to  $CO_2$  increment. For these factors, the  $CO_2$  response was compared with the square root of the increase in  $CO_2$ . For 50% the ratio is 1.5, and therefore the square root is 1.22. Hence photosynthesis or biomass was compared with +22% increase. For stomatal conductance or transpiration, as they were compared as  $A/E$  with 22% i.e.  $A/E = 1.22$ , it also implied  $E/A = 0.82$ , which meant an actual 18% decrease. For 100% the ratio is 2, and therefore the square root is 1.41 and photosynthesis was compared with +41%. Similarly, for transpiration, it was compared as  $A/E$  with 41% i.e.  $A/E = 1.41$  which implied  $E/A = 0.71$ , which meant an actual

29% decrease.

In the second approach, it was tested more directly whether the increase in plant WUE was proportional to increase in  $C_a$ , i.e. it was asked whether

$$\frac{WUE_{\text{elevated}}}{WUE_{\text{ambient}}} = \frac{C_{a \text{ elevated}}}{C_{a \text{ ambient}}} \quad (7)$$

To do this, each observation was normalized by the  $CO_2$  increase applied, modifying equation (5) to be

$$\ln RR = \ln \left( \frac{x_T}{x_C} / \frac{C_{a T}}{C_{a C}} \right) \quad (8)$$

The model predicts that this response ratio (RR) should be equal to one.

To determine whether photosynthesis or transpiration was strongest in determining the change in WUE, a novel analysis was applied which compared the change of each of the variables to the square root of the change in  $C_a$ . For example, for iWUE, equation (8) can be rewritten as

$$\left( \frac{A_T / g_{sT}}{A_C / g_{sC}} \right) / \left( \frac{C_{a T}}{C_{a C}} \right) = 1 \quad (9)$$

Where A denotes photosynthesis and  $g_s$  is stomatal conductance. If photosynthesis and stomatal conductance contribute equally to the change in WUE, then each should change in proportion to the square root of the change in  $C_a$ , photosynthesis increasing and stomatal conductance decreasing. Thus equation (9) was separated so as to obtain separate A and  $g_s$  responses at  $eC_a$ , i.e. for A,

$$RR_A = \left( \frac{A_T}{A_C} \right) / \left( \sqrt{\frac{C_{a T}}{C_{a C}}} \right) \quad (10)$$

Similarly for  $g_s$ ,

$$RR_B = \left( \frac{g_{sC}}{g_{sT}} \right) / \left( \sqrt{\frac{C_{a T}}{C_{a C}}} \right) \quad (11)$$

and tested how these response ratios compared. If the increase in photosynthesis and decrease in stomatal conductance contributed equally to the increase in WUE, then  $RR_A$  should equal  $RR_B$ . If iWUE is proportional to  $C_a$ , then both  $RR_A$  and  $RR_B$  should be equal to 1.

#### 4.2.4 Example:

Here is an example to illustrate how this analysis works. Suppose that at two  $CO_2$  levels, 380ppm and 550ppm ( $CO_2$  increment equal to 45%), the iWUE of a plant increased from 90  $\mu\text{mol } CO_2/\text{mol } H_2O$  to 125  $\mu\text{mol } CO_2/\text{mol } H_2O$  (i.e. an increase of 39%). The



response ratio of plant WUE is slightly less than proportional to the CO<sub>2</sub> increment; the lnRR (equation 8) is equal to -0.04. Suppose the photosynthesis (A) at 380ppm was 9  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and at 550 ppm, 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , an 11% increase. Stomatal conductance ( $g_s$ ) was reduced from 0.1 to 0.08 mol/m<sup>2</sup>/s (from elevated to ambient, this is a 25% increase). Comparing these changes to the CO<sub>2</sub> increment, a response ratio for A of  $\ln(1.11 / \sqrt{1.45}) = -0.08$ , and a response ratio for  $g_s$  of  $\ln(1.25 / \sqrt{1.45}) = +0.04$  would be obtained. These two response ratios sum to the response ratio for iWUE, and indicate in this case that the change in iWUE was more strongly determined by the change in  $g_s$  than by the change in A.

Variances for each equation were calculated similarly to equation (7). Data were categorized into plant photosynthetic pathways (C3 and C4) and plant functional types (C3 and C4herbaceous, angiosperms and gymnosperms). For visualization of the variances within studies, the size of the dots in plots were modified i.e. studies with less variances were more precise and the size of the dot was multiplied with the inverse of the variance. Therefore bigger dots represented more accurate studies. The meta-analysis calculations, forests plots and figures were done using software R (R Development Core Team 2010) with packages ‘metafor’ (Viechtbauer 2010) and ‘multcompView’ (Hothorn et al. 2008).

## 4.3 Results

### 4.3.1 Percent (%) increases in experiments with average 50% increase in CO<sub>2</sub>

Average intrinsic water use efficiency (iWUE), instantaneous transpiration efficiency (iTE) and whole plant water use efficiency (WUE) all increased significantly with increasing CO<sub>2</sub> (Figure 4.1 a, d, g). With an average 50% increase in CO<sub>2</sub>, 50 % increases in iWUE, iTE and WUE were predicted. The overall mean increases in iWUE (56% CI= 37, 78%) and iTE (53% CI= 45, 62%) were not significantly different from this prediction, but the overall mean increase in WUE (33% CI= 29, 37%) was significantly less than 50%.

There were no significant differences among plant functional types (PFTs) in CO<sub>2</sub> effects on iWUE or iTE (Figure 4.1 a, d). iWUE and iTE also responded in proportion to CO<sub>2</sub> for all species. However, WUE responses were less than proportional to CO<sub>2</sub> for C4 herbs and angiosperms while C3 herbs and gymnosperms showed significantly larger responses (Figure 4.1 g).

Examination of the factors contributing to iWUE revealed that on average, increased

photosynthesis (Figure 4.1 b) contributed more to the increase in WUE than the decrease in stomatal conductance or transpiration (Figure 4.1 c). From RR calculations, I predicted an average 22% increase in photosynthesis and 18% decrease in stomatal conductance if both processes were to contribute equally to the change in WUE. The observed average increase in photosynthesis was greater than 22%, while the observed change in stomatal conductance was less than 18%, although both values fall within the calculated 95% CIs. There were no significant differences among PFTs.

Photosynthesis related to iTE (Figure 4.1 e) showed a significantly larger contribution than the related transpiration response (Figure 4.1 f), with the average increase in photosynthesis being significantly larger than 22% (mean=35%, CI=29, 41%) and the average decrease in transpiration being significantly less than 28% (mean=-11%, CI=-15,-6). No significant difference between means of PFTs for photosynthesis were observed. However, for transpiration rates, C3 herb responses were significantly lower than those of C4 herbs and angiosperms.

The average biomass increment (Figure 4.1 h) was not significantly different from 22% (mean=27%, CI=18, 37%). On average the effect in angiosperms being significantly larger than the effect in C4 herbs. Transpirational losses were reduced by  $eC_a$ , but the effect size (Figure 4.1 i) was significantly smaller than the expected value of -18% (mean=-6, CI=-10, -2). No significant difference among means of PFTs were observed.

#### 4.3.2 *Percent (%) increases in experiments with average 100% increase in CO<sub>2</sub>*

A 100% increase in  $C_a$  increased average plant iWUE, iTE and WUE (Figure 4.2 a, d, g), however the % effect was less than proportional for each of these variables. The overall means and 95% CIs for iWUE, iTE and WUE were 76% (CI=60, 93%), 76% (CI=70, 82%) and 48% (CI=41, 55%) respectively. All responded less than proportionally, but WUE had the smallest response compared to iWUE and iTE.

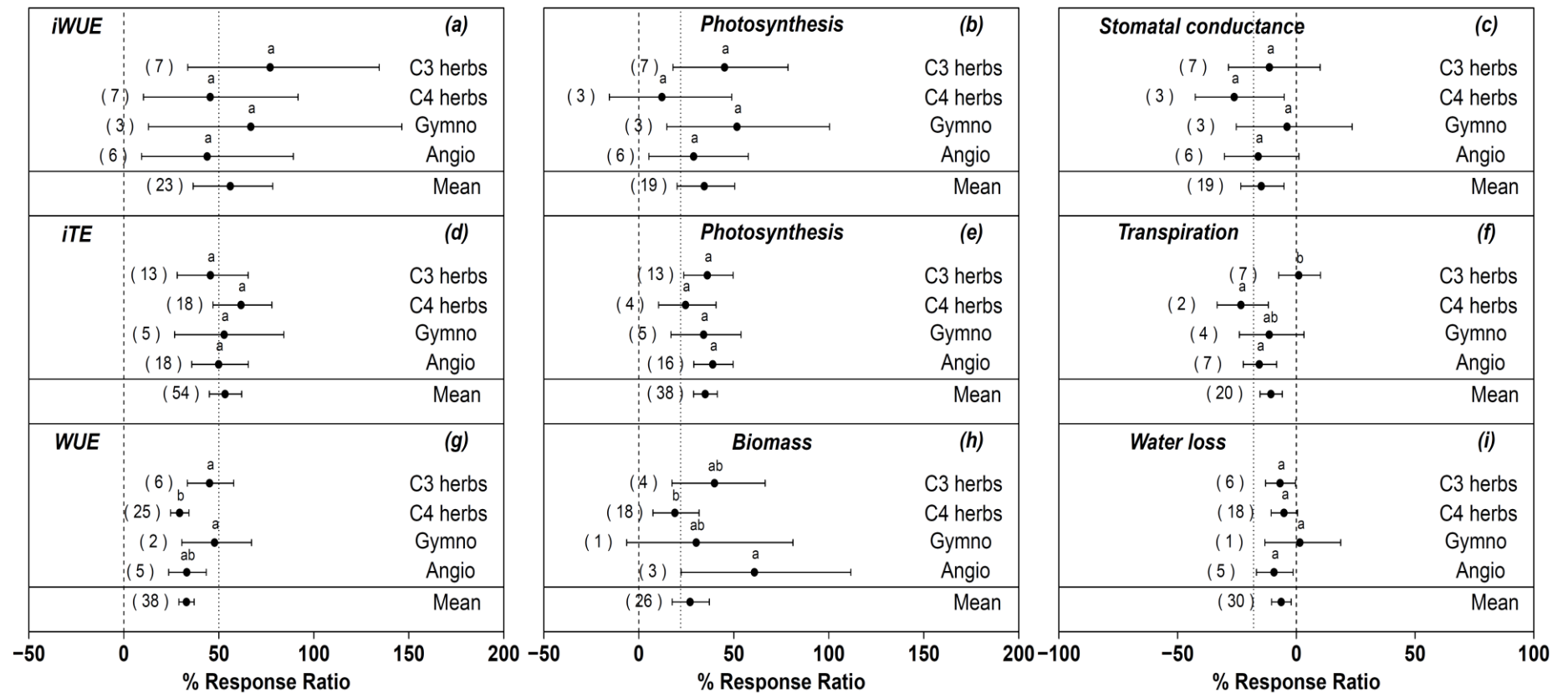
For mean responses of iWUE, there were no significant differences of mean effects among PFTs. The mean iTE response of gymnosperms was significantly less than for other PFTs, whereas at whole-plant scale, the mean CO<sub>2</sub> response of WUE was significantly less than proportional for all PFTs (Figure 4.2 g). C3 herbs had significantly higher WUE than C4 herbs.

For the factors contributing to iWUE, iTE and WUE, a response of 41% for photosynthesis or biomass would be expected (leaf, shoot, above ground or whole plant biomass) and -29% decrease for stomatal conductance or transpirational losses, if each

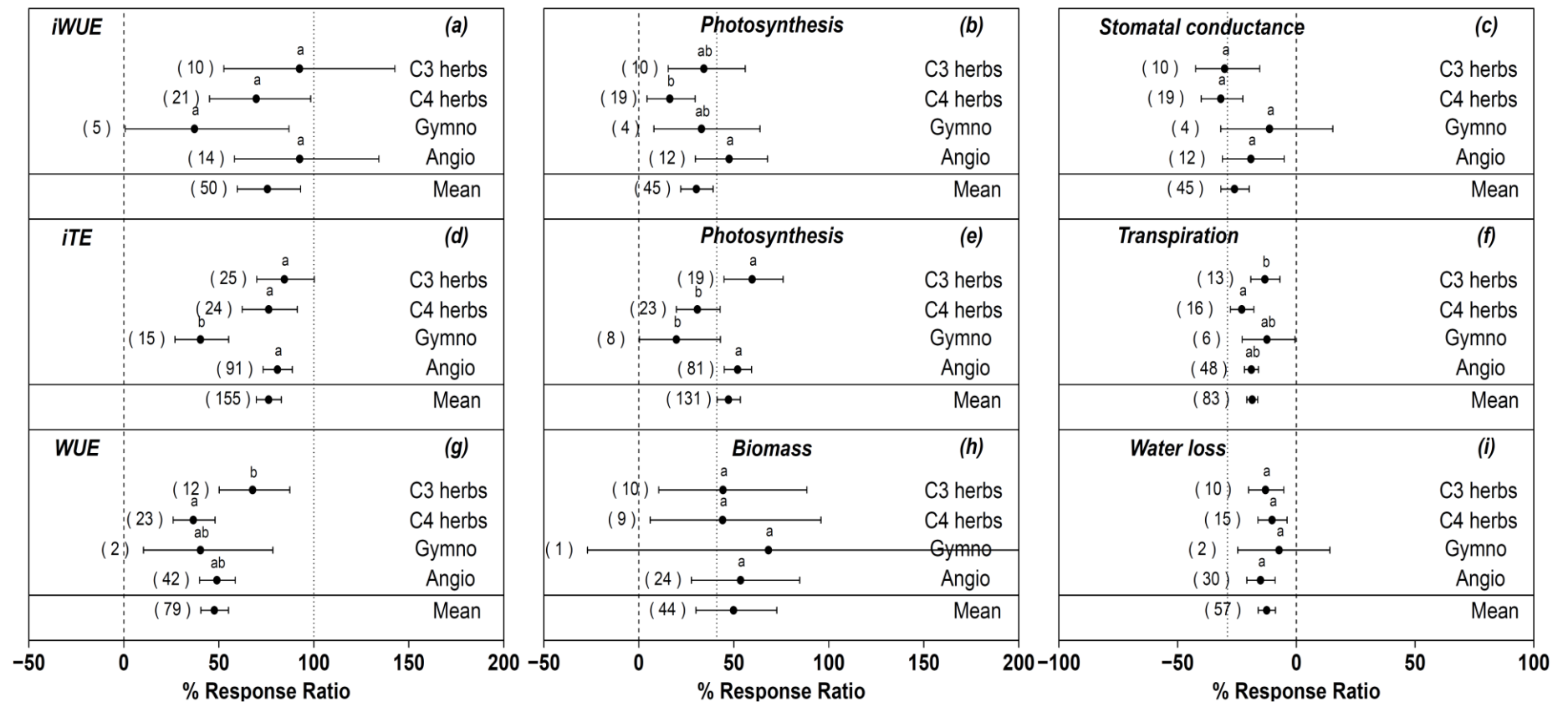
process had contributed equally. Photosynthesis responses related to iWUE were significantly less than +41% (mean=30%, CI=22, 39%) on average, but stomatal conductance responded as predicted (mean=-26, CI=-32, -19). Angiosperms had significantly higher photosynthetic responses when compared to C4 herbs. For stomatal conductance, there was no significant difference among PFTs in their responses, although average responses of C3 and C4 herbs were stronger than those observed in gymnosperms and angiosperms (Figure 4.2 c). Photosynthetic responses related to iTE were significantly higher for C3 herbs and angiosperms than for C4 herbs and gymnosperms (Figure 4.2 e). In contrast, transpirational rates responded significantly more strongly in C4 herbs than for other functional groups (Figure 4.2 f).

At the whole plant scale, there were no significant differences among PFTs for biomass increment (Figure 4.2 h). Similarly there were no significant differences among PFTs for transpiration rates (Figure 4.2 i).

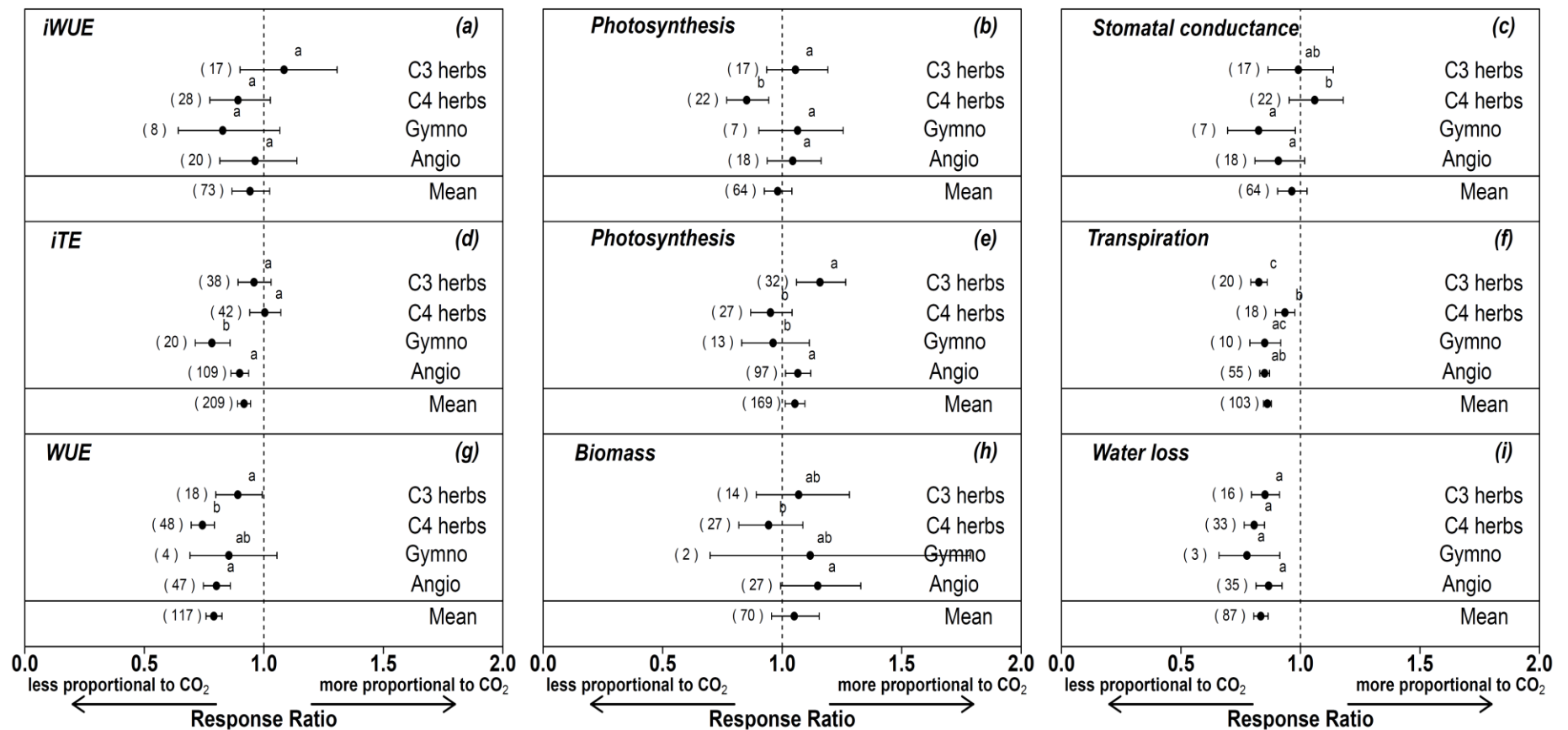
Overall responses indicated that for iWUE, the contributing factors, photosynthesis and stomatal conductance, responded according to the predictions. However, for iTE and WUE values, it was the transpiration rates which responded less than predicted for proportionality to CO<sub>2</sub>. Thereby, smaller increases in iTE and WUE were observed as compared to the increase in iWUE.



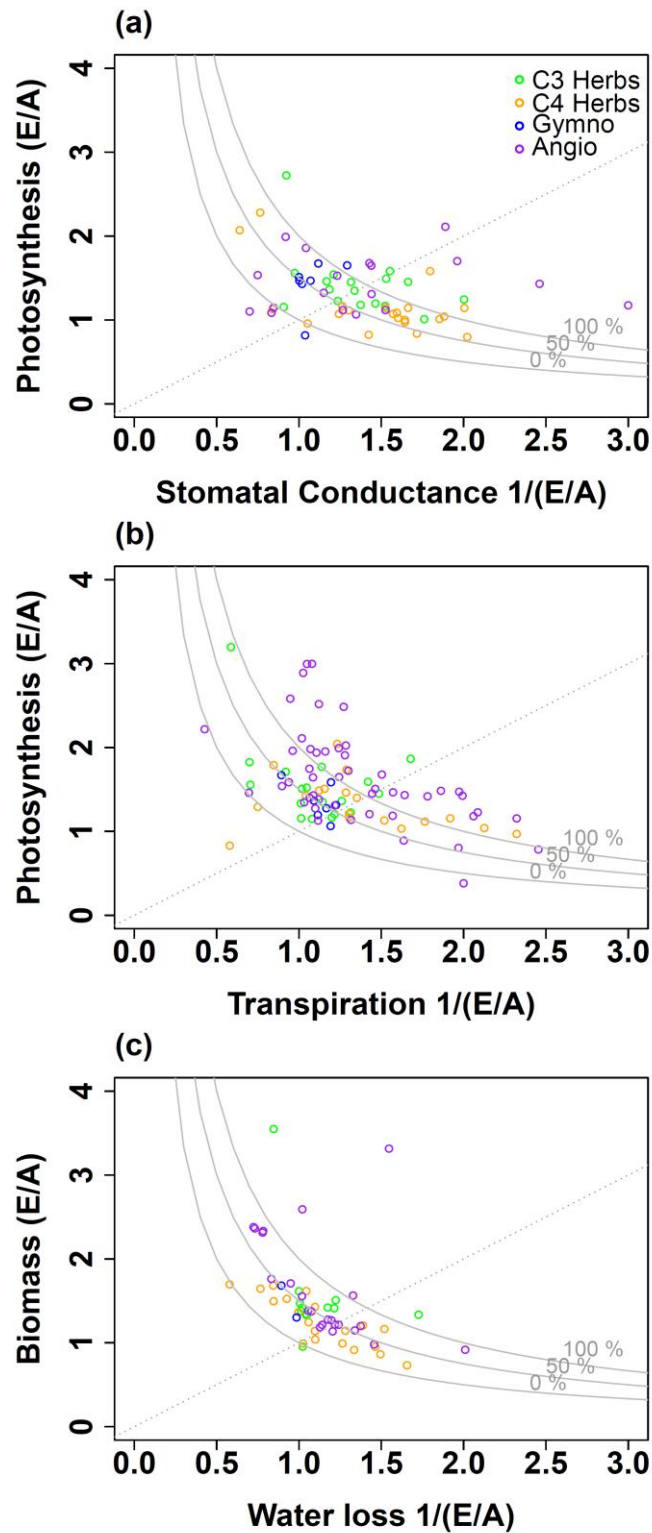
**Figure 4-1:** Meta-analysis of species responses to 50% increase in  $C_a$ . (a) Responses of Intrinsic Water Use Efficiency WUE and the contributing factors Photosynthesis (b) and stomatal conductance (c). Instantaneous Transpiration Efficiency iTE (d) and the contributing factors Photosynthesis (e) and Transpiration rates (f). Whole plant Water Use Efficiency WUE (g), and the contributing factors Biomass increment (h) and water loss through transpiration (i). The dashed vertical lines through zero represent no effect. The dotted vertical lines represent the expected  $CO_2$  effect (50% in (a, d, g), 22% in (b, c, e), and -18% in (c, f, i)). The symbol represents the mean response ( $\pm$  95% CI). The solid horizontal lines represent 95% confidence intervals. The numbers in brackets denote the number of replicates for each study. The significant between group heterogeneity for different functional groups is denoted by small letters. Same letters denote no difference in means ( $p$ -value  $> 0.05$ ).



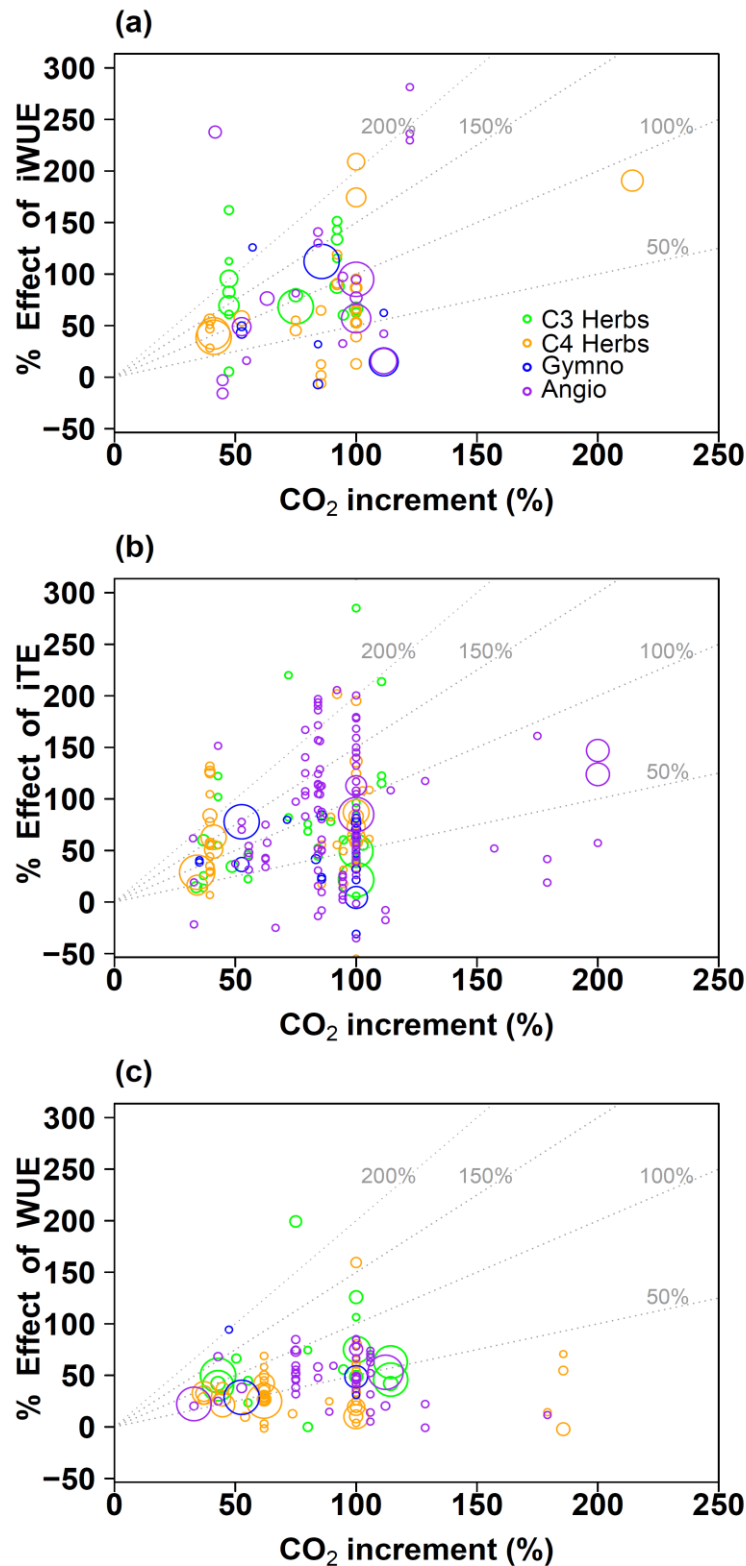
**Figure 4-2:** Meta-analysis of species responses to 100% increase in  $\text{CO}_2$ . (a) Responses of Intrinsic Water Use Efficiency WUE and the contributing factors Photosynthesis (b) and stomatal conductance (c). Instantaneous Transpiration Efficiency iTE (d) and the contributing factors Photosynthesis (e) and Transpiration rates (f). Whole plant Water Use Efficiency WUE (g), and the contributing factors Biomass increment (h) and water loss through transpiration (i). The dashed vertical lines through zero represent no effect. The dotted vertical lines represent the expected  $\text{CO}_2$  effect (100% in (a, d, g), 41% in (b, c, e), and -29% in (c, f, i). The symbol represents the mean response ( $\pm$  95%CI). The solid horizontal lines represent 95% confidence intervals. The numbers in brackets denote the number of replicates for each study. The significant between group heterogeneity for different functional groups is denoted by small letters. Same letters denote no difference in means ( $p$ -value  $> 0.05$ ).



**Figure 4-3:** Meta-analysis of species responses relative to 1:1 increase with  $C_a$ . (a) Responses of Intrinsic Water Use Efficiency WUE and the contributing factors Photosynthesis (b) and stomatal conductance (c). Instantaneous Transpiration Efficiency iTE (d) and the contributing factors Photosynthesis (e) and Transpiration rates (f). Whole plant Water Use Efficiency WUE (g), and the contributing factors Biomass increment (h) and water loss through transpiration (i). The dashed vertical lines through zero represent equal proportionalities. The right arrow represents responses which are more than proportional to  $C_a$  and left arrow represents responses which are less than proportional to  $C_a$ . The symbol represents the mean response ( $\pm$  95%CI). The solid horizontal lines represent 95% confidence intervals. The numbers in brackets denote the number of replicates for each study. The significant between group heterogeneity for different functional groups is denoted by small letters. Same letters denote no difference in means ( $p$ -value  $> 0.05$ ).

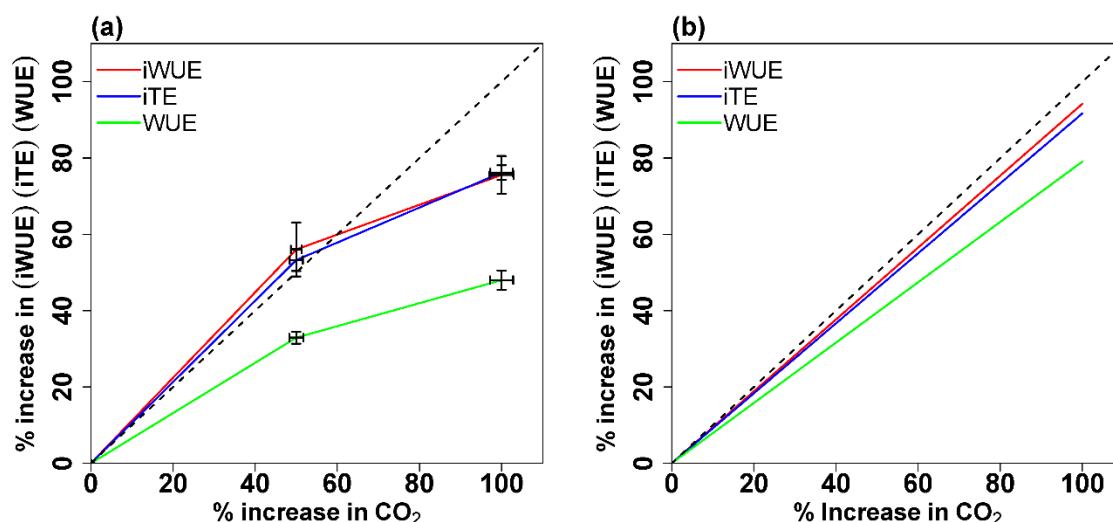


**Figure 4-4:** Plots showing species response ratios to  $eC_a$ . (a) Intrinsic water use efficiency of species. Stomatal conductance ratio (Ambient  $CO_2$ /Elevated  $CO_2$   $A/E$ ) (indicated as  $1/(E/A)$  in the figure) as a function of Photosynthesis ratio ( $E/A$ ). (b) Instantaneous water use efficiency of species. Transpiration ratio ( $A/E$ ) as a function of Photosynthesis ratio ( $E/A$ ). (c) Whole plant water use efficiency. Water loss ratio ( $A/E$ ) as a function of biomass gained ratio ( $E/A$ ). The grey solid lines represent %  $CO_2$  effect. The dotted grey line represents 1:1 line which means equal increase in biomass and water loss. For different colors, see legend in (a).



**Figure 4-5:** Plots showing species % responses to % CO<sub>2</sub> increase. Species responses of (a) intrinsic water use efficiency (iWUE) (b) Instantaneous water use efficiency (iTE) and (c) Whole plant water use efficiency (WUE) to % CO<sub>2</sub> increment. Grey dotted lines represent overall percent effect. The size of the dots presents within study variance. Small dot denotes large variance and less effective study whereas bigger dot denotes small variance and more effective study. For different colors, see legend in (a).





**Figure 4-6:** Overall plants' responses to iWUE, iTE and WUE with proportional increase in  $C_a$ . (a) shows results from first analysis (see methods) where studies were divided into 50% and 100% increase in CO<sub>2</sub> increment. The black bars represents  $\pm$ SE. The dashed line shows 1:1 line. (b) shows results from the second analysis (see methods) for 1:1 relative increase of plant responses to increase in CO<sub>2</sub> concentration. The dashed line represents 1:1 line.

#### 4.3.3 CO<sub>2</sub> Proportionality

For the second analysis it was tested if plant responses were in proportion to CO<sub>2</sub> i.e. whether the slope of a relationship between CO<sub>2</sub> increment and WUE response is equal to 1. Overall responses of iWUE showed 0.94 (CI=0.87, 1.02) proportionality with CO<sub>2</sub> increment and the confidence intervals included the expected value of 1. However for iTE and WUE the responses were less proportional to CO<sub>2</sub>. iTE responses were 0.92 (CI=0.89, 0.94) whereas WUE responses were 0.79 (CI=0.76, 0.82).

At leaf-scale, iWUE responded in proportion to the CO<sub>2</sub> increment (Figure 4.3 a). Although the mean proportionalities of C3 herbs, C4 herbs, gymnosperms and angiosperm were less than 1, their confidence intervals included greater than 1 proportionality. For iTE proportionalities the mean of only gymnosperms was significantly different from other PFTs (Figure 4.3 d). At whole plant scale level, species had WUE proportionalities less than one with respect to CO<sub>2</sub>, however for gymnosperms the confidence intervals included 1 (Figure 4.3 g). The WUE of C4 were significantly lower to C3 herbs and Angiosperms.

For the factors affecting iWUE, iTE and WUE, we hypothesized their change to the square root of the change in CO<sub>2</sub>. The photosynthesis proportionality or mean of C4 herbs was significantly different from rest of the species (Figure 4.3 b). Higher stomatal conductance proportionalities were observed for C3 (mean=0.99, CI=0.86, 1.14) and C4 herbs

(mean=1.06, CI=0.95, 1.18) as compared to gymnosperms (mean=0.82, CI=0.69, 0.98) and angiosperms (mean=0.91, CI=0.81, 1.01) (Figure 4.3 c). The mean proportionality of C4 herbs was significantly different to gymnosperms and angiosperms.

Photosynthesis factor related to  $\dot{V}$ TE showed more than proportional increase to  $\text{CO}_2$  for C3 herbs and angiosperms however, the confidence intervals of C4 herbs and gymnosperms included the expected 1 proportionality (Figure 4.3 e). The means of C3 herbs and angiosperms were significantly different from C4 herbs and gymnosperms. The transpiration rates showed less than proportional responses to  $\text{CO}_2$  (Figure 4.3 f). The C4 herbs had significantly higher transpiration rates compared to C3 herbs.

Biomass increment related to WUE, showed more than a proportional increase for C3 herbs and woody species as compared to C4 herbs (Figure 4.3 h), however for C4 herbs their confidence intervals included the expected proportionality of 1. The mean proportionality of C4 herbs were significantly different from angiosperms. Transpirational losses related to WUE were less than proportional to  $\text{CO}_2$  increase for all the species (Figure 4.3 i). No difference in means was observed among the PFTs.

$\dot{V}$ WUE responses in terms of photosynthesis and stomatal conductance were between 50% and 100% whereas stomatal conductance responses were less (less data below 1:1 line) as compared with photosynthesis (more data above 1:1 line) for all species (Figure 4.4 a). Species  $\dot{V}$ TE responses in terms of photosynthesis and transpiration were around 50 to 100% (Figure 4.4 b). More data were observed above 1:1 line showing that photosynthesis increased more at  $eC_a$  as compared to proportional decrease in transpiration. Similarly, species WUE responses were more congregated around 50% increase suggesting that WUE did not increase in proportion to  $\text{CO}_2$  (Figure 4.4 c). Moreover biomass increment had greater effect as compared to transpirational losses.

Species intrinsic water use efficiency responses with their respective  $\text{CO}_2$  increment showed large variances among studies and more data were observed around 100% increase (grey dotted line) (Figure 4.5 a). Similarly, species instantaneous transpiration efficiency increased in terms of % age with that of  $\text{CO}_2$ . Studies showed large variances, both within and between studies, however the responses were concentrated between 50% increase to 100% increase (Figure 4.5 b). Species responses concentrated around 50% increase for % increase in plant water use efficiency (Figure 4.5 c).

Large variability in studies were observed in the first analysis where studies were categorised into 50% and 100%  $\text{CO}_2$  increment (Figure 4.6 a). For studies which were grouped in 50% increase in  $\text{CO}_2$ , the  $\text{CO}_2$  treatment ranged from 32 -73% for  $\dot{V}$ WUE and  $\dot{V}$ TE,

whereas for WUE it ranged from 39 – 63%. Studies which were grouped in 100% increase in CO<sub>2</sub>, the CO<sub>2</sub> treatment ranged from 75-186%, for iWUE, 75-295% for iTE and 75-214% for WUE. The results showed that at 50% CO<sub>2</sub> increment, iTE and iWUE increased to about 60% with respect to WUE. At 100% CO<sub>2</sub> increment, iTE and iWUE increased to about 64% with respect to WUE. Results of iWUE, iTE and WUE for the second analysis where plant responses were normalised with respective CO<sub>2</sub> concentrations, showed that WUE decreased to 14% with respect to iWUE and iTE (Figure 4.6 b).

## 4.4 Discussion

In the study the chief focus was to test if the plant water use efficiency was proportional to increase in C<sub>a</sub> at leaf and whole plant scale level. The other aspect which I looked at was whether there is difference of responses between different plant functional types.

*Is WUE proportional to CO<sub>2</sub>?*

### *(a) Leaf-scale*

Meta-analysis of the existing literature revealed that with 50% increase in CO<sub>2</sub>, all species overall showed 56% and 53% increase in iWUE and iTE respectively (Figure 4.1a, d), whereas a 100% increase in CO<sub>2</sub> caused only a 76% increase in both iWUE and iTE (Figure 4.2a, d). 1:1 ratio analysis indicated that iWUE and iTE increased in an overall proportion of 0.94 (CI=0.87, 1.02) and 0.92 (CI=0.89, 0.84) across studies, with the iTE response being significantly less than proportional (Figure 4.3a, d). Models predict that A/g<sub>s</sub> should be proportional to CO<sub>2</sub> throughout the range of CO<sub>2</sub>. But the results (from 50% and 100% CO<sub>2</sub> increase) suggests that linearity of the equation ( $WUE \propto CO_2$ ) holds at low CO<sub>2</sub> levels (a 50%) whereas at high CO<sub>2</sub> levels (>50%) the response starts to deviate from linearity (Figure 4.6).

The results also show that at leaf level, iWUE increases in proportion to the increase in C<sub>a</sub> for C<sub>3</sub> herbs, C<sub>4</sub> herbs and woody trees, whereas at whole plant level, WUE is less than proportional to C<sub>a</sub>. The results for mean iTE were close to the mean iWUE (Figure 4.3 a, d), however, the confidence intervals for iWUE included the expected 1 value (CI=0.87, 1.02) whereas the confidence intervals were smaller for iTE (CI=0.89, 0.94). By looking at the factors contributing for iWUE and iTE, stomatal conductances for iWUE were found to be

more responsive to  $C_a$  than transpirational rates. These smaller effects on transpiration rates than on stomatal conductance indicate that responses are related not only to decrease stomatal openings but also to atmospheric feedback effects. While stomatal conductance depends on leaf size, surface structure and stomatal location (Bernacchi et al. 2002), transpiration is dependent on both boundary layer conductance as well as stomatal conductance to operate in series (Smith and Jarvis 1998, Bauerle and Bowden 2011). Their relative magnitude controls which conductance is the leading regulator of transpiration.

In natural environments, as photosynthesis and transpiration increase, changes in transpiration rates at the leaf surface are different from those close to the immediate air when the wind speed over a leaf is less. In artificial environments, however, created by gas exchange equipment, the conditions at leaf surface may vary. The data thus collected by these equipment may not reflect the true natural environmental conditions. The data used in the present analysis were taken using gas exchange cuvettes and may not reflect the true natural environmental conditions. In these cuvettes, the boundary layer conductance is kept high enough to reduce the difference between bulk air and leaf surface conditions to relatively low levels. The differences existing between bulk air and leaf surface conditions may be very significant in terms of feedback mechanisms that regulate stomatal responses. For example in a study by Barton et al. (2012)  $iTE$  was found to be proportional to  $C_a$ , however, it was strongly dependent on  $D$ . It was concluded in the study that differences in  $D$  at both the leaf and canopy level should be taken into account for  $iTE$  measurements. The environment of the leaf is therefore probably best described by considering conditions at the leaf surface. If environmental and gas exchange data are consistent with the conditions at the leaf surface, data obtained from the experiments could provide more meaningful comparison.

#### *(b) Whole plant-scale*

At the whole plant scale, the mean  $CO_2$  response of water use efficiency was significantly less than proportional to the  $CO_2$  increase (Figures 1c, 2c, 3c). With 50% increase in  $CO_2$ , a mean 38% increase in WUE was observed across all functional groups, whereas a 100% increase in  $CO_2$  indicated that species overall showed a mean 48% increase in WUE. Overall, the response of water use efficiency to  $CO_2$  was 0.79 (CI=0.76, 0.82) of the increase in  $C_a$ . Consideration of the factors driving the change in WUE indicated that the reduction in transpirational loss was much smaller than the response of biomass increment. The analysis suggests that the leaf level proportional increase cannot be translated directly to the whole plant scale because negative feedback effects come into force from surface

temperature and atmospheric humidity on the sensitivity of transpiration to a change in leaf conductance. When stomatal conductance is much smaller than boundary layer conductance, stomata are the dominant controller of water loss and a decrease in stomatal conductance will result in a nearly proportional decrease in transpiration. Plants in this state have an omega value near 0 as suggested by Jarvis and McNaughton (1986), and is said to be well coupled. In contrast, when the boundary layer is of similar order of magnitude of stomatal conductance, changes in stomatal conductance will have little effect on transpiration rate, and feedback through radiation to the canopy primarily drive transpiration. In a leaf cuvette, transpiration from individual leaves can be effectively described by the density dynamics. However, transpiration from leaves in a canopy requires consideration of both diffusion and the leaf energy balance.

Boundary layer conductance is determined differently amongst the models. Canopy boundary layer conductance, leaf boundary layer conductance, and/or both are used in calculations and have been described in detail by De Kauwe et al. (2013), along with comparisons of predictions from these models with actual observed data from two FACE sites. De Kauwe et al. (2013), found that overall, model predictions of the CO<sub>2</sub> effect on WUE were more accurate to the data observed at the well-coupled coniferous site (Duke FACE), but were poor at the broadleaf site (Oak Ridge FACE). Moreover, there were discrepancies among models that calculate whole plant water use efficiencies by taking various degrees of plant coupling to the atmosphere. The results of the present study suggest that with 50% increase in C<sub>a</sub> whole plant WUE decrease to 60% whereas with 100% increase in C<sub>a</sub> WUE decreased to 64%. For modelling, it is useful to set a benchmark to enable easier modelling and to compare results. Thus the results of the present study can be used for setting a benchmark for modelling and avoid the discrepancies emerging due to various degrees of the coupling effect incorporated in the models.

#### *Are there any differences among PFTs?*

The study showed that, at leaf scale, C3 and C4 herbs have similar iWUE and iTE responses to elevated CO<sub>2</sub>. The contributing factors for iWUE showed that, the CO<sub>2</sub> effect on photosynthetic rates was comparatively higher in C3 as compared to C4 herbs, whereas stomatal responses were similar for C3 and C4 herbs. The results of this study are comparable to other studies. For example, in a meta-analysis by Ainsworth and Long (2005), a 15% increase of light saturated photosynthetic rate in C4 plants was observed, as compared to a

34% stimulation for C3 plants with increasing CO<sub>2</sub>. Moreover, no difference in the sensitivity of stomatal responses to CO<sub>2</sub> among different C3 and C4 grasses was observed in studies by Morrison and Gifford (1983) and Mehrali et al. (2003). At leaf scale, transpiration is greater in C4 plants and explains the similar responses. At whole plant scale, the water loss responses are smaller than the transpiration responses, which explains why WUE is less responsive in C4 plants than C3 plants (because most of the WUE response comes through transpiration not photosynthesis). Transpiration rates at leaf scale indicated that C4 responses are larger as compared to C3 herbs. However at whole plant scale level there were no difference among species. Accordingly, the results of the present study contradict the general concept that C4 plants due to their already saturated carboxylation efficiencies will respond more in terms of reduced stomatal conductances (Wand et al. 1999, Ainsworth et al. 2002, Sage and Kubien 2003).

At the whole plant scale, species responses to WUE were not different to increasing C<sub>a</sub>. Unexpectedly, no difference in greater biomass accumulation were found for woody species. Trees are generally assumed to be more capable of accumulating biomass as compared to herbaceous species. Trees differ from other type of vegetation in relation to not only storing CO<sub>2</sub> as carbon-rich organic compounds in secondary woody tissues, but also because of their vertical height. Local climates of tall trees are also strongly influenced by heat and water vapour transfer processes which are connected through height of the trees causing proportional vegetation roughness. The roughness indicates the level of coupling of vegetation with the environment and is proportional to height. Moreover, trees also extend their roots deeper than many other type of vegetation and thus can tap additional sources of water (Canadell et al. 1996).

The studies included in the present meta-analysis were conducted on plants with different age and sizes and some studies also have additional treatments such as nutrient, temperature, etc. Responses of plants can differ under varying environmental condition. Hence, large variances among studies were observed in the analysis. Small number of studies on gymnosperms rendered low power to the analysis and larger confidence intervals were observed. Moreover, many experimental studies from literature do not provide the essential statistical information about the results needed for them to be included in meta-analytical studies and therefore, the present study is not exhaustive.

One of today's scientific challenges is to directly link the observations at ecosystem levels to develop a profound understanding of biotic interactions with environmental constraints. The present study only provides an overview of WUE changes at leaf and plant-

scale. Scaling from plants to ecosystems, and from short to long timescales involves system-level experimentation and modelling. Additional analyses are beyond the scope of the present study, but the analyses at ecosystem scale can deliver better information for global scale models. For example, analysing eddy covariance data can provide further insight in the coupling of carbon and water fluxes in a range of ecosystems. Further research may analyze water fluxes of the whole ecosystems, which essentially govern the response of ecosystems to changing environmental conditions. Analysing such plant properties from globally up-scaled flux fields may help to provide distinct spatial patterns.

### *Conclusion*

In summary, the results suggest that plant water use efficiency shows a close linear relation with increasing CO<sub>2</sub> only at the leaf-scale. However, scaling up to whole plant or canopy level, plant water use efficiencies showed less than proportional increases because the transpiration rates may be reduced due to a boundary layer effect. At the whole plant level, WUE decreased to 60% with respect to Leaf-level iWUE and iTE with 50% CO<sub>2</sub> increment, whereas at 100% CO<sub>2</sub> increment WUE decreased to 64% with respect to iWUE and iTE. Moreover, at leaf-scale level no differences between plant functional groups' means were found for stomatal conductance. Transpiration rates were decreased in C4 herbaceous species, however, at whole plant scale the responses were similar to the other species. At whole plant scale level, water use efficiencies were less than proportional to CO<sub>2</sub>. Due to large variability in the data no difference was found among functional groups. In natural environments, the plant transpiration response may vary due to coupling effect. Unnatural ventilation in growth cabinets, glasshouses and open-top chambers provide poor coupling of plants to the atmosphere and thus may not accurately reflect CO<sub>2</sub> effects on transpiration of natural vegetation. Models predicting CO<sub>2</sub> effect on whole plant use efficiencies may correct uncertainties by considering the changes arising at whole plant scale level due to coupling or decoupling of leaf boundary layer with atmosphere.

### **References**

- Ainsworth, E. A., P. A. Davey, C. J. Bernacchi, O. C. Dermody, E. A. Heaton, D. J. Moore, P. B. Morgan, S. L. Naidu, H. S. Y. Ra, X. G. Zhu, P. S. Curtis, and S. P. Long. 2002. A meta-analysis of elevated [CO<sub>2</sub>] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology* 8:695-709.
- Ainsworth, E. A. and S. P. Long. 2005. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytologist* 165:351-372.

- Ball, J. T., I. Woodrow, and J. Berry. 1987. A Model Predicting Stomatal Conductance and its Contribution to the Control of Photosynthesis under Different Environmental Conditions. Pages 221-224 in J. Biggins, editor. Progress in Photosynthesis Research. Springer Netherlands.
- Ball, M. C., M. J. Cochrane, and H. M. Rawson. 1997. Growth and water use of the mangroves *Rhizophora apiculata* and *R-stylosa* in response to salinity and humidity under ambient and elevated concentrations of atmospheric CO<sub>2</sub>. Plant Cell and Environment 20:1158-1166.
- Bange, G. G. J. 1953. On the quantitative explanation of stomatal transpiration. Acta Botanica Neerlandica 2:255-297.
- Barton, C. V. M., R. A. Duursma, B. E. Medlyn, D. S. Ellsworth, D. Eamus, D. T. Tissue, M. A. Adams, J. Conroy, K. Y. Crous, M. Liberloo, M. Low, S. Linder, and R. E. McMurtrie. 2012. Effects of elevated atmospheric [CO<sub>2</sub>] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. Global Change Biology 18:585-595.
- Bauerle, W. L. and J. D. Bowden. 2011. Separating foliar physiology from morphology reveals the relative roles of vertically structured transpiration factors within red maple crowns and limitations of larger scale models. Journal of Experimental Botany 62:4295-4307.
- Bernacchi, C. J., A. R. Portis, H. Nakano, S. von Caemmerer, and S. P. Long. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiology 130:1992-1998.
- Brakke, M. and L. H. Allen. 1995. Gas-Exchange of Citrus Seedlings at Different Temperatures, Vapor-Pressure Deficits, and Soil-Water Contents. Journal of the American Society for Horticultural Science 120:497-504.
- Brodribb, T. J., S. A. M. McAdam, G. J. Jordan, and T. S. Feild. 2009. Evolution of stomatal responsiveness to CO<sub>2</sub> and optimization of water-use efficiency among land plants. New Phytologist 183:839-847.
- Bryant, J., G. Taylor, and M. Frehner. 1998. Photosynthetic acclimation to elevated CO<sub>2</sub> is modified by source : sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. Plant Cell and Environment 21:159-168.
- Bunce, J. A. 2004. Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. Oecologia 140:1-10.
- Canadell, J. G., L. F. Pitelka, and J. S. I. Ingram. 1996. The effects of elevated [CO<sub>2</sub>] on plant-soil carbon below-ground: A summary and synthesis. Plant and Soil 187:391-400.
- Cao, B., Q. L. Dang, and S. R. Zhang. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO<sub>2</sub>] in white birch seedlings. Tree Physiol 27:891-899.
- Centritto, M., M. E. Lucas, and P. G. Jarvis. 2002. Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach (*Prunus persica*) seedlings in response to elevated carbon dioxide concentration and water availability. Tree Physiol 22:699-706.
- Cernusak, L. A., K. Winter, C. Martinez, E. Correa, J. Aranda, M. Garcia, C. Jaramillo, and B. L. Turner. 2011. Responses of Legume Versus Nonlegume Tropical Tree Seedlings to Elevated CO<sub>2</sub> Concentration. Plant Physiology 157:372-385.
- Curtis, P. S. and X. Z. Wang. 1998. A meta-analysis of elevated CO<sub>2</sub> effects on woody plant mass, form, and physiology. Oecologia 113:299-313.
- Damour, G., T. Simonneau, H. Cochard, and L. Urban. 2010. An overview of models of stomatal conductance at the leaf level. Plant Cell and Environment 33:1419-1438.
- De Kauwe, M. G., B. E. Medlyn, S. Zaehle, A. P. Walker, M. C. Dietze, T. Hickler, A. K. Jain, Y. Q. Luo, W. J. Parton, I. C. Prentice, B. Smith, P. E. Thornton, S. S. Wang, Y. P. Wang, D. Warland, E. S. Weng, K. Y. Crous, D. S. Ellsworth, P. J. Hanson, H. Seok Kim, J. M. Warren, R. Oren, and R. J. Norby. 2013. Forest water use and water use efficiency at elevated CO<sub>2</sub>: a model-data intercomparison at two contrasting temperate forest FACE sites. Global Change Biology 19:1759-1779.
- De Luis, I., J. J. Irigoyen, and M. Sanchez-Diaz. 1999. Elevated CO<sub>2</sub> enhances plant growth in droughted N-2-fixing alfalfa without improving water status. Physiologia Plantarum 107:84-89.



- Drake, B. G., M. A. González-Meler, and S. P. Long. 1997. MORE EFFICIENT PLANTS: A Consequence of Rising Atmospheric CO<sub>2</sub>? Annual Review of Plant Physiology and Plant Molecular Biology 48:609-639.
- Eamus, D. and P. G. Jarvis. 1989. The Direct Effects of Increase in the Global Atmospheric CO<sub>2</sub> Concentration on Natural and Commercial Temperate Trees and Forests. Advances in Ecological Research 19:1-55.
- Franks, P. J., P. L. Drake, and D. J. Beerling. 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. Plant, Cell & Environment 32:1737-1748.
- Geller, G. N. and W. K. Smith. 1982. Influence of Leaf Size, Orientation, and Arrangement on Temperature and Transpiration in 3 High-Elevation, Large-Leafed Herbs. Oecologia 53:227-234.
- Ghannoum, O., S. V. Caemmerer, L. H. Ziska, and J. P. Conroy. 2000. The growth response of C4 plants to rising atmospheric CO<sub>2</sub> partial pressure: a reassessment. Plant, Cell & Environment 23:931-942.
- Ghannoum, O., N. G. Phillips, M. A. Sears, B. A. Logan, J. D. Lewis, J. P. Conroy, and D. T. Tissue. 2010. Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO<sub>2</sub>] and temperature. Plant Cell and Environment 33:1671-1681.
- Gifford, R. M. 1995. Whole plant respiration and photosynthesis of wheat under increased CO<sub>2</sub> concentration and temperature: Long-term vs short-term distinctions for modelling. Global Change Biology 1:385-396.
- Greenep, H., M. H. Turnbull, and D. Whitehead. 2003. Response of photosynthesis in second-generation *Pinus radiata* trees to long-term exposure to elevated carbon dioxide partial pressure. Tree Physiol 23:569-576.
- Haworth, M., C. Elliott-Kingston, and J. C. McElwain. 2011. Stomatal control as a driver of plant evolution. Journal of Experimental Botany 62:2419-2423.
- Heath, O. V. S. 1948. Control of stomatal movement by a reduction in the normal carbon dioxide content of the air.
- Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80:1150-1156.
- Hetherington, A. M., and F. I. Woodward. 2003. The role of stomata in sensing and driving environmental change. Nature 424:901-908.
- Holmes, W. E., D. R. Zak, K. S. Pregitzer, and J. S. King. 2006. Elevated CO<sub>2</sub> and O<sub>3</sub> alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. Ecosystems 9:1354-1363.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50:346-363.
- Hovenden, M. J. and A. L. Williams. 2010. The impacts of rising CO<sub>2</sub> concentrations on Australian terrestrial species and ecosystems. Austral Ecology 35:665-684.
- Hughes, L. 2000. Biological consequences of global warming: is the signal already apparent? Trends in Ecology & Evolution 15:56-61.
- IPCC. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.
- Jarvis, P. G. 1985. Transpiration and assimilation of tree and agricultural crops: the "omega factor". Pages 460-480 in Cannell and J. E. Jackson, editors. Attributes of trees as crop plants. Institute of Terrestrial Ecology, UK.
- Jarvis, P. G. and K. G. McNaughton. 1986. Stomatal Control of Transpiration - Scaling up from Leaf to Region. Advances in Ecological Research 15:1-49.
- Kimball, B. A., J. R. Mauney, F. S. Nakayama, and S. B. Idso. 1993. Effects of increasing atmospheric CO<sub>2</sub> on vegetation. Vegetatio 104-105:65-75.
- King, J. S., M. E. Kubiske, K. S. Pregitzer, G. R. Hendrey, E. P. McDonald, C. P. Giardina, V. S. Quinn, and D. F. Karnosky. 2005. Tropospheric O<sub>3</sub> compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO<sub>2</sub>. New Phytologist 168:623-636.

- Knapp, A. K., E. P. Hamerlynck, J. M. Ham, and E. O. Clenton. 1996. Responses in Stomatal Conductance to Elevated CO<sub>2</sub> in 12 Grassland Species That Differ in Growth Form. *Vegetatio* 125:31-41.
- Koike, T., T. T. Lei, T. C. Maximov, R. Tabuchi, K. Takahashi, and B. I. Ivanov. 1996. Comparison of the photosynthetic capacity of Siberian and Japanese birch seedlings grown in elevated CO<sub>2</sub> and temperature. *Tree Physiol* 16:381-385.
- Korner, C. 2000. Biosphere responses to CO<sub>2</sub> enrichment. *Ecological Applications* 10:1590-1619.
- Kubiske, M. E. and K. S. Pregitzer. 1997. Ecophysiological responses to simulated canopy gaps of two tree species of contrasting shade tolerance in elevated CO<sub>2</sub>. *Functional Ecology* 11:24-32.
- Lajeunesse, M. J. 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology* 92:2049-2055.
- Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C<sub>3</sub> plants. *Plant, Cell & Environment* 18:339-355.
- Li, C. R., L. J. Gan, K. Xia, X. Zhou, and C. S. Hew. 2002. Responses of carboxylating enzymes, sucrose metabolizing enzymes and plant hormones in a tropical epiphytic CAM orchid to CO<sub>2</sub> enrichment. *Plant, Cell & Environment* 25:369-377.
- Long, S. P., E. A. Ainsworth, A. Rogers, and D. R. Ort. 2004. Rising atmospheric carbon dioxide: Plants face the future. *Annual Review of Plant Biology* 55:591-628.
- Long, S. P. and B. G. Drake. 1992. Photosynthetic CO<sub>2</sub> assimilation and rising atmospheric CO<sub>2</sub> concentrations. Pages 69-103 in N. R. B. a. H. Thomas., editor. *Crop Photosynthesis: Spatial and Temporal Determinants*. Elsevier Science Publishers B.V., Amsterdam.
- Maherali, H., H. B. Johnson, and R. B. Jackson. 2003. Stomatal sensitivity to vapour pressure difference over a subambient to elevated CO<sub>2</sub> gradient in a C<sub>3</sub>/C<sub>4</sub> grassland. *Plant Cell and Environment* 26:1297-1306.
- Martin, T. A., T. M. Hinckley, F. C. Meinzer, and D. G. Sprugel. 1999. Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. *Tree Physiol* 19:435-443.
- Medlyn, B. E., C. V. M. Barton, M. S. J. Broadmeadow, R. Ceulemans, P. De Angelis, M. Forstreuter, M. Freeman, S. B. Jackson, S. Kellomaki, E. Laitat, A. Rey, P. Roberntz, B. D. Sigurdsson, J. Strassmeyer, K. Wang, P. S. Curtis, and P. G. Jarvis. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO<sub>2</sub> concentration: a synthesis. *New Phytologist* 149:247-264.
- Medlyn, B. E., R. A. Duursma, D. Eamus, D. S. Ellsworth, I. C. Prentice, C. V. M. Barton, K. Y. Crous, P. de Angelis, M. Freeman, and L. Wingate. 2011. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17:2134-2144.
- Monteith, J. L. and H. Unsworth. 1990. *Principles of Environmental Physics*. Edward Arnold.
- Morgan, J. A., D. R. LeCain, A. R. Mosier, and D. G. Milchunas. 2001. Elevated CO<sub>2</sub> enhances water relations and productivity and affects gas exchange in C<sub>3</sub> and C<sub>4</sub> grasses of the Colorado shortgrass steppe. *Global Change Biology* 7:451-466.
- Morison, J. I. L. 1993. Response of Plants to CO<sub>2</sub> under Water Limited Conditions. *Vegetatio* 104:193-209.
- Morison, J. I. L. and R. M. Gifford. 1983. Stomatal Sensitivity to Carbon-Dioxide and Humidity - a Comparison of 2 C<sub>3</sub> and 2 C<sub>4</sub> Grass Species. *Plant Physiology* 71:789-796.
- NOAA. 2011. National Climatic Data Center, State of the Climate: Global Analysis for Annual
- Nobel, P. S. 1991. *Physicochemical and Environmental Plant Physiology*. Elsevier Science.
- Norby, R. J., E. H. DeLucia, B. Gielen, C. Calfapietra, C. P. Giardina, J. S. King, J. Ledford, H. R. McCarthy, D. J. P. Moore, R. Ceulemans, P. De Angelis, A. C. Finzi, D. F. Karnosky, M. E. Kubiske, M. Lukac, K. S. Pregitzer, G. E. Scarascia-Mugnozza, W. H. Schlesinger, and R. Oren. 2005. Forest response to elevated CO<sub>2</sub> is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences of the United States of America* 102:18052-18056.
- Nowak, R. S., D. S. Ellsworth, and S. D. Smith. 2004. Functional responses of plants to elevated atmospheric CO<sub>2</sub> - do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* 162:253-280.

- Overdieck, D. and M. Forstreuter. 1994. Evapotranspiration of Beech Stands and Transpiration of Beech Leaves Subject to Atmospheric CO<sub>2</sub> Enrichment. *Tree Physiol* 14:997-1003.
- Pataki, D. E., T. E. Huxman, D. N. Jordan, S. F. Zitzer, J. S. Coleman, S. D. Smith, R. S. Nowak, and J. R. Seemann. 2000. Water use of two Mojave Desert shrubs under elevated CO<sub>2</sub>. *Global Change Biology* 6:889-897.
- Picon, C., J. M. Guehl, and G. Aussenac. 1996. Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO<sub>2</sub> and drought. *Annales Des Sciences Forestieres* 53:431-446.
- Poorter, H. 1993. Interspecific Variation in the Growth-Response of Plants to an Elevated Ambient CO<sub>2</sub> Concentration. *Vegetatio* 104:77-97.
- Poorter, H. and M. L. Navas. 2003. Plant growth and competition at elevated CO<sub>2</sub>: on winners, losers and functional groups. *New Phytologist* 157:175-198.
- R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robinson, J. M. 1994. Speculations on Carbon-Dioxide Starvation, Late Tertiary Evolution of Stomatal Regulation and Floristic Modernization. *Plant Cell and Environment* 17:345-354.
- Sage, R. F. and D. S. Kubien. 2003. Quo vadis C4? An ecophysiological perspective on global change and the future of C4 plants. *Photosynthesis Research* 77:209-225.
- Saxe, H., D. S. Ellsworth, and J. Heath. 1998. Tree and forest functioning in an enriched CO<sub>2</sub> atmosphere. *New Phytologist* 139:395-436.
- Schuepp, P. H. 1993. Tansley Review No. 59 Leaf boundary layers. *New Phytologist* 125:477-507.
- Smith, D. M. and P. G. Jarvis. 1998. Physiological and environmental control of transpiration by trees in windbreaks. *Forest Ecology and Management* 105:159-173.
- Talhelm, A. F., K. S. Pregitzer, and C. P. Giardina. 2012. Long-Term Leaf Production Response to Elevated Atmospheric Carbon Dioxide and Tropospheric Ozone. *Ecosystems* 15:71-82.
- Taneva, L., J. S. Phippen, W. H. Schlesinger, and M. A. Gonzalez-Meler. 2006. The turnover of carbon pools contributing to soil CO<sub>2</sub> and soil respiration in a temperate forest exposed to elevated CO<sub>2</sub> concentration. *Global Change Biology* 12:983-994.
- Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO<sub>2</sub> and temperature. *Tree Physiol* 18:715-726.
- Tschaplinski, T. J., D. B. Stewart, P. J. Hanson, and R. J. Norby. 1995. Interactions between Drought and Elevated CO<sub>2</sub> on Growth and Gas-Exchange of Seedlings of 3 Deciduous Tree Species. *New Phytologist* 129:63-71.
- Viechtbauer, W. 2010. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software* 36:1-48.
- Vu, J. C. V. 2005. Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO<sub>2</sub> and temperature. *Environmental and Experimental Botany* 53:85-95.
- Ward, S. J. E., G. F. Midgley, M. H. Jones, and P. S. Curtis. 1999. Responses of wild C4 and C3 grass (*Poaceae*) species to elevated atmospheric CO<sub>2</sub> concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* 5:723-741.
- Wayne, P. M., E. G. Reekie, and F. A. Bazzaz. 1998. Elevated CO<sub>2</sub> ameliorates birch response to high temperature and frost stress: implications for modeling climate-induced geographic range shifts. *Oecologia* 114:335-342.
- Wertin, T. M., M. A. McGuire, and R. O. Teskey. 2010. The influence of elevated temperature, elevated atmospheric CO<sub>2</sub> concentration and water stress on net photosynthesis of loblolly pine (*Pinus taeda* L.) at northern, central and southern sites in its native range. *Global Change Biology* 16:2089-2103.
- Wullschlegel, S. D., C. A. Gunderson, P. J. Hanson, K. B. Wilson, and R. J. Norby. 2002. Sensitivity of stomatal and canopy conductance to elevated CO<sub>2</sub> concentration - interacting variables and perspectives of scale. *New Phytologist* 153:485-496.
- Zak, D. R., M. E. Kubiske, K. S. Pregitzer, and A. J. Burton. 2012. Atmospheric CO<sub>2</sub> and O<sub>3</sub> alter competition for soil nitrogen in developing forests. *Global Change Biology* 18:1480-1488.
- Zak, D. R., K. S. Pregitzer, M. E. Kubiske, and A. J. Burton. 2011. Forest productivity under elevated CO<sub>2</sub> and O<sub>3</sub>: positive feedbacks to soil N cycling sustain decade-long net primary productivity enhancement by CO<sub>2</sub>. *Ecology Letters* 14:1220-1226.

Zheng, Y. R., Z. X. Xie, G. M. Rimmington, Y. J. Yu, Y. Gao, G. S. Zhou, P. An, X. J. Li, W. Tsuji, and H. Shimizu. 2010. Elevated CO<sub>2</sub> accelerates net assimilation rate and enhance growth of dominant shrub species in a sand dune in central Inner Mongolia. *Environmental and Experimental Botany* 68:31-36.

## CHAPTER 5

### Discussion and Conclusions

Extensive research on the effects of elevated  $\text{CO}_2$  ( $eC_a$ ) on ecosystem structure and function has demonstrated that modifications in gas exchange and growth are among the primary responses of vegetation to the current rise in atmospheric  $\text{CO}_2$  concentration ( $C_a$ ). Ecophysiological models estimate predicted vegetation growth under rising  $\text{CO}_2$  and climate change, based on assumptions about the interaction of plant physiological processes and the environment. Any change in the climate drivers ( $\text{CO}_2$ , temperature and precipitation) cause new situations that can add inherent uncertainty in the models. Models based on physiological processes may contain further uncertainties from experimental errors and from the assumptions incorporated into the models. Therefore, uncertainties are increased when models are scaled up to the level of stands and ecosystems.

It is important that these scaled up models are regularly tested and are based on current empirical knowledge. The practice will not only improve our knowledge of plant functioning and but will also advance research where interactions between plants and climate change have to be taken into consideration. This study attempts to link new experimental findings with model formulations and to test the underlying assumptions in the models.

One of the assumptions made in the models is that plant responses at  $eC_a$  should be higher at higher temperatures. The assumption is made at leaf-level (Long 1991) and at canopy scale models (Mcmurtrie and Wang 1993). However, some models do not incorporate this important assumption. To date, numerous studies have been conducted on plant growth responses to  $eC_a$  and temperature. It is unclear if these studies support the above assumption made by the models. I used meta-analysis technique to find out if there is truly an interaction between  $eC_a$  and temperature.

In chapter 2, I carried out two meta-analyses. Firstly I collected all the factorial  $eC_a \times$  temperature experiments on trees from literature to find an interaction, and secondly, I collected all the  $\text{CO}_2$  experiments carried out all over the globe on trees to find a relationship of plant responses to their mean annual temperatures. The first meta-analysis results showed that there is a positive interaction between  $eC_a$  and high temperature, however the results were not significant as the confidence intervals were too large to identify the positive effect. The second regression-meta-analysis results indicated that the plant responses were stronger at

warm tropics as compared to boreal forest. However, a lack of studies contributed to a non-significant effect of mean annual temperature with plant growth responses. Hence, neither of the meta-analyses allowed to differentiate between presence or absence of  $eC_a$  x temperature interaction.

The issues with the analysis were, firstly there was high variability in the data and secondly, there were limited amount of studies which reported the results with statistical summaries (e.g., standard errors, number of replicates). It is important that studies convey their results in a form that is consistent and could be used for re-analysis purposes. The study also emphasizes the need for further experiments investigating  $CO_2$  responses in the specific regions-boreal and tropics.

From modelling prospective, it would be useful to estimate plant responses to climate change equally with and without incorporating the  $eC_a$  x temperature interaction until further data become available.

In chapter 3, firstly I tested whether the optimal stomatal conductance model proposed by Medlyn et al. (2011) can be used to predict stomatal behavior of C4 species. One of the assumptions made in the model was that the C3 plants respond as to be optimizing for RuBP-regeneration limiting reaction rather than Rubisco limiting reaction. The model has been tested to work well with C3 species. However, its accuracy for C4 species is unknown because of the underlying assumptions in the model. Secondly, I tested whether C4 stomata are more responsive to  $eC_a$  compared to C3 species. It is assumed that due to their already saturated assimilation rates, C4 species will respond more strongly in terms of closing their stomates to rising  $C_a$ .

To test these hypotheses, a glasshouse experiment was designed to measure responses of C3 and C4 species to change in environmental factors. Congeneric C3 and C4 species were selected with well-defined C3 and C4 traits. The results of the study showed that the optimal stomatal conductance model by Medlyn et al. (2011) accurately predicted the C4 responses. The model provides  $g_1$  values (the slope of the model  $g_s$  vs.  $A C_a \sqrt{D}$ ) which can be used as a proxy to calculate  $\lambda$ , the marginal carbon cost of water ( $\text{mol H}_2\text{O mol}^{-1} \text{C}$ ). The values of  $\lambda$  for C3 species were found to be similar to that of C4 species. However the  $g_1$  values of C4 were found to be approximately half of those of C3 species. Lower  $g_1$  values were observed because of the saturated nature of the A-Ci curves of C4 plants. The similar  $\lambda$  values in both C3 and C4 species indicate that C4 species have not changed their marginal carbon cost of water. The results imply that C4 species have evolved to maximize their assimilation rates by

keeping similar carbon cost of water compared to C3 species. Moreover, the study did not find any evidence that the stomata of C4 species are more sensitive to CO<sub>2</sub> than C3 species.

The model by Medlyn et al. (2011) provides a framework to interpret stomatal responses for both C3 and C4 species to changes in environmental factors. The study also shows that numerical solution of the optimal stomatal behaviour model does not accurately predict  $g_s$  responses of C4 species.

In chapter 4, I tested model predictions that plants should increase their water use efficiencies in proportion to an increase in CO<sub>2</sub> at leaf-level and at whole plant scale level. The models also predict that the proportional increase is uniform across all plant functional types. Again, I used a meta-analysis technique to test if the present data from experimental outcome support these model assumptions. The results show that the predictions are true at leaf-scale however, the proportionality decreases when the responses are scaled up to whole plant level. Overall responses indicated that for leaf-level iWUE (intrinsic water use efficiency =  $A/g_s$ ), the contributing factors, photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ), responded according to the predictions i.e. proportional increase with CO<sub>2</sub>. However, for iTE (instantaneous water use efficiency =  $A/E$ ) and whole plant WUE (water use efficiency =  $\text{Biomass}/E$ ), it was the transpiration rates ( $E$ ) which responded less than proportionally to CO<sub>2</sub>. Thereby, a lower increase in iTE and WUE was observed as compared to iWUE values. With a 50% increase in CO<sub>2</sub>, the response of WUE was only about 60% of the response of iWUE and iTE, whereas with 100% increase in CO<sub>2</sub>, the response in WUE was about 64% of the response of iWUE and iTE.

It was inferred from the study that the “coupling effect” plays an important role in determining whole plant water use efficiency (WUE). The more strongly plants are coupled to the atmosphere (i.e. greater roughness is observed in the boundary layer which is adjacent to the plant or leaf surface) the more the stomata have control over transpiration (Jarvis and Mcnaughton 1986). In de-coupled state a reduction in stomatal conductance does not result in proportional decrease in transpiration. Hence, model predictions that WUE increases in proportion to  $C_a$  are accurate at leaf-scale and match well with the measured data. These data are taken in closed cuvettes of gas-exchange analyzers. The cuvettes may provide a perfect coupling of the leaf to its surrounding, however in natural environments the leaf responses tend to deviate from linearity due to de-coupling effect. It is thus, important to consider the boundary layer effects in models to accurately reflect CO<sub>2</sub> effects on transpiration of natural vegetation.

The analysis on plant functional type revealed that the CO<sub>2</sub> effect on whole-plant WUE in C4 plants was statistically less than the CO<sub>2</sub> effect on WUE in C3 plants. At leaf scale there was no difference in the effect on iWUE or iTE but the change originated less from photosynthesis and more from transpiration in C4 plants. At the canopy scale, changes in transpiration were diminished more than changes in photosynthesis, due to the coupling effect. That elucidated the fact why WUE of C4 plants was less responsive than that of C3 plants.

From modelling point of view, it is important that models incorporate the boundary layer effects occurring at leaf and canopy-scales. However, to tackle the discrepancies shown by the models, it is useful to set a quantitative benchmark against which to compare results. Setting a standard metric for model evaluation will help to assess the reliability of vegetation models. This study thus, puts forward a benchmark data set for comparing and analyzing model performance as well as providing data for model development.

To enable better predictions of the climate effects on vegetation, it is important to incorporate novel information on the potential impacts of different climate change scenarios on plant growth into models. The research on the potential effects of climate change on plant growth is vast. However, it is important that the experiments are designed in a way that is useful for modelers and that the data reported can be incorporated into model predictions. Improving model predictions is important to guide policy makers and managers in sustaining and managing ecosystems affected by climate change.

## References

- Jarvis, P. G. and K. G. Mcnaughton. 1986. Stomatal Control of Transpiration - Scaling up from Leaf to Region. *Advances in Ecological Research* 15:1-49.
- Long, S. P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO<sub>2</sub> concentrations: Has its importance been underestimated? *Plant, Cell & Environment* 14:729-739.
- Mcmurtrie, R. E. and Y. P. Wang. 1993. Mathematical-Models of the Photosynthetic Response of Tree Stands to Rising CO<sub>2</sub> Concentrations and Temperatures. *Plant Cell and Environment* 16:1-13.
- Medlyn, B. E., R. A. Duursma, D. Eamus, D. S. Ellsworth, I. C. Prentice, C. V. M. Barton, K. Y. Crous, P. de Angelis, M. Freeman, and L. Wingate. 2011. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17:2134-2144.



## **APPENDICES**

Appendix A:

Table A1: A list of CO<sub>2</sub> x temperature experiments giving mean values with standard errors and number of replicates. For abbreviations refer to abbreviation list at the end of table A2.

References	Location	Parameter	Parameter Units	Species	Functional Division	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Units	Facility	Interacting treatment	Temp1	Temp2	LCLT	LCLTSd	LCLTN	HCLT	HCLTSd	HCLTN	LCHT	LCHTSd	LCHTN	HCHT	HCHTSd	HCHTN
Olszyk et al., 2003	NHEEL, OR, USA	RTWT	g	<i>Pseudotsuga menziesii</i>	Gymno	350	530	μmol/mol	GC	None	Ambient	Ambient + 3.5 °C	186.99	15.05	3	186.95	0.00	3	186.95	45.17	3	191.30	15.05	3
Olszyk et al., 2003	NHEEL, OR, USA	ABGRWT	g	<i>Pseudotsuga menziesii</i>	Gymno	350	530	μmol/mol	GC	None	Ambient	Ambient + 3.5 °C	365.24	195.79	3	343.78	97.90	3	3295.65	75.29	3	3113.04	90.36	3
Olszyk et al., 2003	NHEEL, OR, USA	TW	g	<i>Pseudotsuga menziesii</i>	Gymno	350	530	μmol/mol	GC	None	Ambient	Ambient + 3.5 °C	547.82	218.38	3	321.73	120.48	3	3478.26	128.02	3	3500.00	112.95	3
Overdieck et al., 2007	Berlin, Germany	TW	g	<i>Fagus Sylvatica</i>	Gymno	400	700	μmol/mol	GH	None	Ambient -2 °C	Ambient + 2 °C	99.39	12.19	9	89.09	20.33	9	9112.12	13.55	9	9126.06	14.89	9
Overdieck et al., 2007	Berlin, Germany	TW	g	<i>Fagus Sylvatica</i>	Gymno	400	700	μmol/mol	GH	None	Ambient	Ambient + 4 °C	76.96	17.62	9	90.30	9.48	9	9129.09	12.19	9	9139.39	9.48	9
Delucia et al., 1997	Duke University, NC, USA	TW	g	<i>Pinus ponderosa</i>	Gymno	350	700	μL/L	GC	None	25/10 °C	30/15 °C	20.00	10.19	24	30.26	10.53	24	25.52	9.85	24	30.33	15.14	24
Delucia et al., 1997	Duke University, NC, USA	RTWT	g	<i>Pinus ponderosa</i>	Gymno	350	700	μL/L	GC	None	25/10 °C	30/15 °C	10.89	10.19	24	14.65	10.53	24	11.19	9.85	24	14.84	15.14	24
Delucia et al., 1997	Duke University, NC, USA	ABGRWT	g	<i>Pinus ponderosa</i>	Gymno	350	700	μL/L	GC	None	25/10 °C	30/15 °C	9.11	10.19	24	15.61	10.53	24	14.33	9.85	24	15.49	15.14	24
Callaway et al., 1994	Duke University, NC, USA	TW	g	<i>Pinus ponderosa</i>	Gymno	350	650	μbar	GC	None	10/25 °C	15/30 °C	1.66	0.16	5	1.76	0.16	5	1.70	0.18	5	2.15	0.20	5
Callaway et al., 1994	Duke University, NC, USA	RTWT	g	<i>Pinus ponderosa</i>	Gymno	350	650	μbar	GC	None	10/25 °C	15/30 °C	0.79	0.16	5	0.83	0.16	5	0.75	0.18	5	1.01	0.20	5
Callaway et al., 1994	Duke University, NC, USA	ABGRWT	g	<i>Pinus ponderosa</i>	Gymno	350	650	μbar	GC	None	10/25 °C	15/30 °C	0.91	0.16	5	0.93	0.16	5	0.95	0.18	5	1.14	0.20	5
Uleman et al., 2000	Duke University, NC, USA	RTWT	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	13.85	8.63	8	15.61	6.68	8	15.33	7.04	8	17.64	6.36	8
Uleman et al., 2000	Duke University, NC, USA	TW	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	23.54	15.96	8	28.19	14.63	8	36.28	12.41	8	40.62	14.63	8
Uleman et al., 2000	Duke University, NC, USA	ABGRWT	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	9.69	8.63	8	12.58	6.68	8	20.95	7.04	8	22.98	6.36	8
Uleman et al., 2000	Duke University, NC, USA	RTWT	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	3.69	1.98	8	6.04	4.78	8	5.30	3.59	8	4.93	3.37	8
Uleman et al., 2000	Duke University, NC, USA	TW	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	7.69	4.44	8	10.74	8.42	8	16.33	7.54	8	15.80	8.87	8
Uleman et al., 2000	Duke University, NC, USA	ABGRWT	g	<i>Robinia pseudoacacia</i>	Angio	35	70	Pa	GC	Nitrogen	26 °C	30 °C	4.00	1.98	8	4.70	4.78	8	11.03	3.59	8	10.87	3.37	8
King et al., 1996	Duke University, NC, USA	RTWT	g	<i>Pinus taeda</i>	Gymno	35	70	Pa	GH	Fertilized	Ambient	Ambient + 5 °C	6.13	1.07	5	10.05	3.29	5	5.77	2.55	5	12.92	2.33	5
King et al., 1996	Duke University, NC, USA	RTWT	g	<i>Pinus taeda</i>	Gymno	35	70	Pa	GH	Fertilized	Ambient	Ambient + 5 °C	4.19	0.56	5	8.45	1.79	5	3.58	1.72	5	11.64	2.59	5
King et al., 1996	Duke University, NC, USA	RTWT	g	<i>Pinus ponderosa</i>	Gymno	35	70	Pa	GH	Fertilized	Ambient	Ambient + 5 °C	4.98	2.01	5	9.22	1.61	5	5.41	1.86	5	10.66	2.93	5
King et al., 1996	Duke University, NC, USA	RTWT	g	<i>Pinus ponderosa</i>	Gymno	35	70	Pa	GH	Fertilized	Ambient	Ambient + 5 °C	4.13	1.12	5	10.54	2.86	5	4.76	0.92	5	8.88	2.84	5
Sgurdsson et al., 2013	Flakaliden, Sweden	STWT	g/tree	<i>Picea Abies</i>	Gymno	365	700	μmol/mol	WTC	None	Ambient	Ambient + 2.8 °C	521.28	44.22	3	393.62	55.28	3	3508.51	99.50	3	3585.11	210.06	3
Wayne et al., 1998	Hanvard, MA, USA	TW	g	<i>Betula aleghaniensis</i>	Angio	400	800	μL/L	GC	None	Ambient	31/26 °C	4.07	1.10	18	6.36	2.29	18	1.10	0.55	18	3.66	1.27	18
Bruhn et al., 2000	DNA, Denmark	RTWT	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient -2 °C	Ambient + 2.3 °C	2.16	1.30	9	3.36	1.25	9	2.23	0.92	9	4.33	2.58	9
Bruhn et al., 2000	DNA, Denmark	ABGRWT	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient -2 °C	Ambient + 2.3 °C	4.66	6.94	9	3.77	1.83	9	4.05	1.40	9	6.51	3.69	9
Bruhn et al., 2000	DNA, Denmark	TW	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient -2 °C	Ambient + 2.3 °C	6.82	7.84	9	7.13	2.91	9	6.27	2.26	9	10.84	6.10	9
Bruhn et al., 2000	DNA, Denmark	RTWT	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient	Ambient + 4.8 °C	2.34	1.00	9	2.94	1.97	9	1.55	0.67	9	3.57	1.66	9
Bruhn et al., 2000	DNA, Denmark	ABGRWT	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient	Ambient + 4.8 °C	3.27	1.37	9	3.60	1.98	9	2.75	1.16	9	5.09	1.30	9
Bruhn et al., 2000	DNA, Denmark	TW	g	<i>Fagus Sylvatica</i>	Gymno	40	74	Pa	CTC	None	Ambient	Ambient + 4.8 °C	5.61	2.31	9	6.54	3.78	9	4.30	1.78	9	8.66	2.72	9
Kuokkanen et al., 2001	MRS, Finland	TW	g	<i>Betula Pendula</i>	Gymno	360	720	ppm	CTC	None	Ambient	Ambient + 2 °C	5.57	1.36	4	6.27	0.77	4	4.80	1.95	4	7.77	2.05	4
Kelomaki and Wang, 2001	MRS, Finland	TW	g	<i>Betula Pendula</i>	Gymno	360	700	μmol/mol	CTC	None	Ambient	Ambient + 2.5 °C	21.90	5.37	8	25.70	4.24	8	27.40	9.05	8	26.00	5.66	8
Salas et al., 2003	MRS, Finland	ABGRWT	g	<i>Pinus Sylvestris</i>	Gymno	325	732	ppm	GC	None	19/12 °C	23/16 °C	3.20	0.49	6	3.40	0.98	6	4.80	0.98	6	5.50	0.98	6
Veteli et al., 2002	MRS, Finland	ABGRWT	g	<i>Salix myrsinifolia</i>	Gymno	360	720	ppm	CTC	None	Ambient	Ambient + 2 °C	11.05	2.10	4	12.48	1.71	4	10.67	2.67	4	15.81	2.67	4
Norby and Luo, 2004	ORNL, TN, USA	STWT	Kg	<i>Acer Rubrum</i>	Angio	300	600	μmol/mol	OTC	None	18 °C	22 °C	3.92	0.82	3	7.14	2.10	3	2.59	0.68	3	5.61	0.61	3
Norby and Luo, 2004	ORNL, TN, USA	STWT	Kg	<i>Acer Saccharum</i>	Angio	300	600	μmol/mol	OTC	None	18 °C	22 °C	3.80	0.61	3	3.25	0.61	3	2.39	0.54	3	3.80	0.61	3
Wan et al., 2004	ORNL, TN, USA	RTWT	g/m <sup>2</sup>	<i>Acer rubrum/saccharum</i>	Gymno	Ambient CO <sub>2</sub>	Ambient + 300	ppm	OTC	None	Ambient	Ambient + 4 °C	96.62	6.22	3	102.01	32.96	3	39.15	11.19	3	74.71	39.82	3
Wan et al., 2004	ORNL, TN, USA	ABGRWT	g/m <sup>2</sup>	<i>Acer rubrum/saccharum</i>	Gymno	Ambient CO <sub>2</sub>	Ambient + 300	ppm	OTC	None	Ambient	Ambient + 4 °C	88.56	6.22	3	94.86	32.96	3	37.02	11.19	3	70.64	39.82	3
Ghannoum et al., 2010	Richmond, Australia	STWT	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	20.79	4.80	9	29.70	4.36	9	31.67	4.39	9	39.35	4.06	9
Ghannoum et al., 2010	Richmond, Australia	RTWT	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	24.01	6.62	9	29.18	9.85	9	29.63	10.50	9	35.39	7.66	9
Ghannoum et al., 2010	Richmond, Australia	TW	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	65.80	12.65	9	88.65	14.22	9	91.83	14.71	9	112.59	7.99	9
Ghannoum et al., 2010	Richmond, Australia	STWT	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	3.89	1.96	9	8.69	2.69	9	8.47	2.94	9	15.24	3.73	9
Ghannoum et al., 2010	Richmond, Australia	RTWT	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	5.77	3.52	9	11.30	3.03	9	7.80	3.44	9	15.27	6.09	9
Ghannoum et al., 2010	Richmond, Australia	TW	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	14.75	6.29	9	29.44	6.23	9	24.01	8.19	9	46.33	13.21	9
Mortenson, 1996	Saerheim Research Station, Norway	RTWT	g	<i>Betula pubescens</i>	Angio	350	700	μmol/mol	GC	None	15 °C	20 °C	0.59	0.04	2	0.62	0.01	2	0.66	0.01	2	0.71	0.03	2
Mortenson, 1996	Saerheim Research Station, Norway	ABGRWT	g	<i>Betula pubescens</i>	Angio	350	700	μmol/mol	GC	None	15 °C	20 °C	0.84	0.01	2	0.89	0.03	2	1.01	0.06	2	1.24	0.11	2
Mortenson, 1996	Saerheim Research Station, Norway	TW	g	<i>Betula pubescens</i>	Angio	350	700	μmol/mol	GC	None	15 °C	20 °C	3.40	0.06	2	3.74	0.04	2	3.83	0.14	2	4.77	0.20	2
Hou et al., 2010	Shanghai, China	TW	g	<i>Abies faxoniana</i>	Gymno	Ambient CO <sub>2</sub>	350	μmol/mol	GC	None	Ambient	Ambient + 2 °C	58.94	5.83	6	71.38	7.79	6	77.28	8.11	6	82.28	8.16	6
Hou et al., 2010	Shanghai, China	RTWT	g	<i>Abies faxoniana</i>	Gymno	Ambient CO <sub>2</sub>	350	μmol/mol	GC	None	Ambient	Ambient + 2 °C	17.43	5.83	6	22.82	7.79	6	25.41	8.11	6	22.66	8.16	6
Hou et al., 2010	Shanghai, China	ABGRWT	g	<i>Abies faxoniana</i>	Gymno	Ambient CO <sub>2</sub>	350	μmol/mol	GC	None	Ambient	Ambient + 2 °C	41.51	5.83	6	48.56	7.79	6	51.87	8.11	6	59.62	8.16	6
Sheu and Lin, 1999	Taichung, Taiwan	TW	g	<i>Shima superba</i>	Angio	375	720	ppm	GC	None	25/20 °C	30/25 °C	11.80	2.49	12	13.40	10.57	12	14.50	3.64	12	21.60	7.62	12
Usami et al., 2001	Ibaraki, Japan	ABGRWT	g	<i>Quercus myrsinaefolia</i>	Angio	400	768	μmol/mol	GC	None	15.5 °C	17.3 °C	79.00	16.55	16	102.00	19.24	16	78.00	14.76	16	126.00	19.24	16
Usami et al., 2001	Ibaraki, Japan	RTWT	g	<i>Quercus myrsinaefolia</i>	Angio	400	768	μmol/mol	GC	None	15.5 °C	17.3 °C	56.00	17.00	16	82.00	25.00	16	50.00	16.00	16	85.00	20.00	16
Usami et al., 2001	Ibaraki, Japan	TW	g	<i>Quercus myrsinaefolia</i>	Angio	400	768	μmol/mol	GC	None	15.5 °C	17.3 °C	134.20	36.70	16	184.50	45.10	16	128.80	32.00	16	210.70	38.60	16

References	Location	Parameter	Parameter Units	Species	Functional Division	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Units	Facility	Interacting treatment	Temp1	Temp2	LCLT	LCLTSa	LCLTN	HCLT	HCLTSa	HCLTN	LCHT	LCHTSa	LCHTN	HCHT	HCHTSa	HCHTN
Maherali and Delucia, 2000	Urbana, IL, USA	1W	g	<i>Pinus ponderosa</i>	Gymno	350	750	μmol/mol	GC	None	25/15 °C	30/20 °C	4.03	1.80	10	5.15	1.56	10	4.25	1.42	10	5.33	2.32	10
Maherali and Delucia, 2000	Urbana, IL, USA	RTWT	g	<i>Pinus ponderosa</i>	Gymno	350	750	μmol/mol	GC	None	25/15 °C	30/20 °C	2.24	1.80	10	3.00	1.56	10	2.32	1.42	10	2.97	2.32	10
Maherali and Delucia, 2000	Urbana, IL, USA	STWT	g	<i>Pinus ponderosa</i>	Gymno	350	750	μmol/mol	GC	None	25/15 °C	30/20 °C	1.79	1.80	10	2.15	1.56	10	1.93	1.42	10	2.36	2.32	10
Lewis et al., 2013	Richmond, Australia	RTWT	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	12.60	2.20	4	16.10	2.60	4	13.60	2.60	4	17.00	7.40	4
Lewis et al., 2013	Richmond, Australia	ABGRWT	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	22.20	1.80	4	30.20	3.40	4	27.60	5.80	4	33.90	4.00	4
Lewis et al., 2013	Richmond, Australia	1W	g	<i>Eucalyptus saligna</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	34.80	3.80	4	46.30	4.20	4	41.20	7.60	4	50.80	9.00	4
Lewis et al., 2013	Richmond, Australia	RTWT	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	5.50	1.00	4	9.40	1.20	4	5.40	1.80	4	12.30	3.60	4
Lewis et al., 2013	Richmond, Australia	ABGRWT	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	13.90	2.60	4	17.80	3.60	4	13.20	1.80	4	22.60	6.40	4
Lewis et al., 2013	Richmond, Australia	1W	g	<i>Eucalyptus sideroxylon</i>	Angio	400	650	μL/L	GH	None	26/18 °C	30/22 °C	19.40	3.60	4	27.20	4.20	4	18.60	3.40	4	34.90	4.40	4
Duan et al., 2013	Richmond, Australia	RTWT	g	<i>Eucalyptus globulus</i>	Angio	400	640	μL/L	GH	None	28/17 °C	32/21 °C	16.79	1.62	6	15.41	0.04	6	17.11	0.01	6	15.46	0.96	6
Duan et al., 2013	Richmond, Australia	STWT	g	<i>Eucalyptus globulus</i>	Angio	400	640	μL/L	GH	None	28/17 °C	32/21 °C	14.40	2.12	6	12.49	0.05	6	14.86	0.02	6	12.55	1.27	6
Duan et al., 2013	Richmond, Australia	1W	g	<i>Eucalyptus globulus</i>	Angio	400	640	μL/L	GH	None	28/17 °C	32/21 °C	53.86	6.56	6	48.09	0.17	6	55.21	0.06	6	48.27	3.91	6
Bauweraerts et al., 2013	GA, USA	1W	g	<i>Quercus rubra</i>	Angio	385	704	μmol/mol	OTC	None	Ambient	Ambient+ 3 °C	82.47	23.11	3	104.49	23.11	3	100.90	24.12	3	113.48	24.12	3
Lavola et al., 2013	MRS, Finland	ABGRWT	g	<i>Betula pendula</i>	Angio	360	700	μmol/mol	CTC	None	Ambient	Ambient + 2.5 °C	2.92	1.62	288	3.50	1.62	288	3.36	1.62	288	3.83	1.62	288
Lavola et al., 2013	MRS, Finland	RTWT	g	<i>Betula pendula</i>	Angio	360	700	μmol/mol	CTC	None	Ambient	Ambient + 2.5 °C	0.63	0.43	288	0.83	0.32	288	0.77	0.32	288	0.82	0.32	288
Lavola et al., 2013	MRS, Finland	1W	g	<i>Betula pendula</i>	Angio	360	700	μmol/mol	CTC	None	Ambient	Ambient + 2.5 °C	3.51	0.65	288	4.24	1.94	288	4.08	0.65	288	4.58	1.94	288
Ijoelker et al., 1998	MN, USA	1W	g	<i>Picea mariana</i>	Gymno	370	580	μmol/mol	GC	None	21/15	24/19	0.44	0.20	6	1.43	0.52	4	0.48	0.23	6	0.93	0.31	6
Ijoelker et al., 1998	MN, USA	1W	g	<i>Picea mariana</i>	Gymno	370	580	μmol/mol	GC	None	21/21	30/24	0.40	0.16	5	0.70	0.26	6	0.22	0.10	6	0.15	0.04	6
Ijoelker et al., 1998	MN, USA	1W	g	<i>Pinus banksiana</i>	Gymno	370	580	μmol/mol	GC	None	21/16	24/19	0.29	0.05	4	0.70	0.37	4	0.62	0.34	7	0.50	0.18	6
Ijoelker et al., 1998	MN, USA	1W	g	<i>Pinus banksiana</i>	Gymno	370	580	μmol/mol	GC	None	27/22	30/25	1.55	0.84	4	1.75	0.88	3	0.23	0.11	6	0.47	0.14	5
Ijoelker et al., 1998	MN, USA	1W	g	<i>Larix laricina</i>	Angio	370	580	μmol/mol	GC	None	21/17	24/20	2.36	1.48	4	2.18	1.00	4	1.35	0.81	6	1.68	0.27	5
Ijoelker et al., 1998	MN, USA	1W	g	<i>Larix laricina</i>	Angio	370	580	μmol/mol	GC	None	27/23	30/26	1.28	0.20	4	2.39	0.31	3	1.37	0.80	3	1.30	0.39	4
Ijoelker et al., 1998	MN, USA	1W	g	<i>Betula papyrifera</i>	Angio	370	580	μmol/mol	GC	None	21/18	24/21	11.72	4.45	4	14.08	5.83	4	10.69	2.88	3	8.07	1.74	4
Ijoelker et al., 1998	MN, USA	1W	g	<i>Betula papyrifera</i>	Angio	370	580	μmol/mol	GC	None	27/24	30/27	14.57	7.42	4	20.59	10.35	4	11.51	3.25	2	13.23	4.98	3

Table A2: A List of experiments of elevated CO<sub>2</sub> responses of trees freely rooted in the ground. For abbreviations refer to abbreviation list at the end of the table.

References	Site/Location	Type of Experiment	Biomass/ NPP	Parameter Units	Nutrients	Time of Exposure	MAI °C	Lat	Long	Species	Functional Division	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	m2l	Sea	sd2l	n2l	m1l	See	sd1l	n1l	Mean E/A	nRR
Smith et al., 2013	Bangore, Wales, UK	FACE	Biomass	g/m <sup>2</sup>	None	1460	10.2	53 11 N	-04 10 W	<i>Alnus glutinosa</i>	N2-fixing	Ambient	580	ppm	5146.89	251.75	503.50	4	6741.26	125.87	251.74	4	1.31	0.27
Smith et al., 2013	Bangore, Wales, UK	FACE	Biomass	g/m <sup>2</sup>	None	1460	10.2	53 11 N	-04 10 W	<i>Betula pendula</i>	Angio	Ambient	580	ppm	6993.01	629.37	1258.74	4	7790.21	125.87	251.74	4	1.11	0.11
Smith et al., 2013	Bangore, Wales, UK	FACE	Biomass	g/m <sup>2</sup>	None	1460	10.2	53 11 N	-04 10 W	<i>Fagus sylvatica</i>	Angio	Ambient	580	ppm	804.20	117.48	234.96	4	879.72	96.50	193.01	4	1.09	0.09
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	H-N	1460	9.5	47 21 N	08 26 W	<i>Fagus sylvatica</i>	Angio	370	570	μmol/mol	1323.97	104.16	208.32	4	1198.97	52.08	104.16	4	0.91	-0.10
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	H-N	1460	9.5	47 21 N	08 26 W	<i>Picea abies</i>	Gymno	370	570	μmol/mol	1938.55	72.92	145.84	4	2271.88	83.34	166.68	4	1.17	0.16
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	Low-N	1460	9.5	47 21 N	08 26 W	<i>Fagus sylvatica</i>	Angio	370	570	μmol/mol	917.72	72.92	145.83	4	688.54	41.68	83.33	4	0.75	-0.29
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	Low-N	1460	9.5	47 21 N	08 26 W	<i>Picea abies</i>	Gymno	370	570	μmol/mol	1532.30	114.58	229.16	4	1886.47	114.59	229.16	4	1.23	0.21
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	H-N	1460	9.5	47 21 N	08 26 W	<i>Fagus sylvatica</i>	Angio	370	570	μmol/mol	1995.32	104.16	208.32	4	2339.07	125.00	250.00	4	1.17	0.16
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	H-N	1460	9.5	47 21 N	08 26 W	<i>Picea abies</i>	Gymno	370	570	μmol/mol	1771.36	93.75	187.50	4	2417.21	177.10	354.20	4	1.36	0.31
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	Low-N	1460	9.5	47 21 N	08 26 W	<i>Fagus sylvatica</i>	Angio	370	570	μmol/mol	2089.07	114.61	229.22	4	2526.57	270.84	541.68	4	1.21	0.19
Spinnler et al., 2002	Birmendorf, Switz	OTC	Biomass	g/m <sup>2</sup>	Low-N	1460	9.5	47 21 N	08 26 W	<i>Picea abies</i>	Gymno	370	570	μmol/mol	1833.86	62.50	125.00	4	2417.19	177.10	354.20	4	1.32	0.28
Roden et al., 1999	Bungendore, Aus	OTC	Biomass	g	None	225	12.7	-35 14 S	149 26 E	<i>Eucalyptus pauciflora</i>	Angio	350	A+350	uL/L	21.49	1.78	6.89	15	33.00	3.56	13.79	15	1.54	0.43
Loveys et al 2010	Bungendore, Aus	OTC	Biomass	g	None	307	12.7	-35 14 S	149 26 E	<i>Eucalyptus pauciflora</i>	Angio	Ambient	652	μmol/mol	74.28	9.29	20.76	5	120.00	12.86	28.75	5	1.62	0.48
Barker et al., 2005	Bungendore, Aus	OTC	Biomass	g	None	250	12.7	-35 14 S	149 26 E	<i>Eucalyptus pauciflora</i>	Angio	Ambient	2xambient	μmol/mol	13.31	1.05	2.35	5	8.85	1.84	4.11	5	0.66	-0.41
Goodfellow et al., 1997	Darwin, Aus	CTC	Biomass	Kg	None	850	27.2	-12 36 S	131 09 E	<i>Mangifera indica</i>	Angio	Ambient	700	μmol/mol	1.49	0.14	0.39	8	2.39	0.19	0.53	8	1.60	0.47
McCarthy et al. 2010	Durham, NC, USA	FACE	NPP	g/m <sup>2</sup> /yr	None	3650	15.3	35 58 N	-79 05 W	<i>Pinus taeda</i>	Gymno	350	550	μmol/mol	2122.85	97.93	169.62	3	2722.61	182.64	316.35	3	1.28	0.25
Tissue et al., 1997	Durham, NC, USA	OTC	Biomass	g	None	1460	15.3	35 58 N	-79 05 W	<i>Pinus taeda</i>	Gymno	35	65	Pa	12811.56	1121.94	1943.26	3	24481.04	2465.10	4269.67	3	1.91	0.65
Rey and Jarvis, 1997	Glencorse, UK	OTC	Biomass	g	Intmd-N	1324	8.3	55 31 N	-03 12 W	<i>Betula pendula</i>	Angio	350	700	μmol/mol	10.15	0.52	1.26	6	14.91	0.77	1.88	6	1.47	0.38
Temperton et al., 2003	Glendevon, UK	OTC	Biomass	g	None	361	8.1	56 12 N	-3 36 W	<i>Alnus glutinosa</i>	Angio	350	700	μmol/mol	219.01	25.04	50.08	4	223.03	18.40	36.80	4	1.02	0.02
Temperton et al., 2003	Glendevon, UK	OTC	Biomass	g	None	361	8.1	56 12 N	-3 36 W	<i>Alnus glutinosa</i>	Angio	350	700	μmol/mol	142.10	28.35	56.69	4	216.10	20.34	40.68	4	1.52	0.42
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	361	8.1	56 12 N	-3 36 W	<i>Betula pendula</i>	Angio	350	700	μmol/mol	93.65	28.23	56.46	4	100.04	14.70	29.40	4	1.07	0.07
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	361	8.1	56 12 N	-3 36 W	<i>Betula pendula</i>	Angio	350	700	μmol/mol	43.48	18.96	37.91	4	45.41	2.72	5.44	4	1.04	0.04
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	1457	8.1	56 12 N	-03 36W	<i>Pinus sylvestris</i>	Angio	350	700	μmol/mol	1089.90	206.86	413.71	4	1737.80	264.05	528.10	4	1.59	0.47
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	1457	8.1	56 12 N	-03 36W	<i>Pinus sylvestris</i>	Angio	350	700	μmol/mol	713.40	111.49	222.98	4	1288.50	284.15	568.30	4	1.81	0.59
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	726	8.1	56 12 N	-03 36W	<i>Picea sitchensis</i>	Angio	350	700	μmol/mol	772.90	146.45	292.90	4	1693.70	364.84	729.68	4	2.19	0.78
Laitat et al., 1999	Glendevon, UK	OTC	Biomass	g	None	726	8.1	56 12 N	-03 36W	<i>Picea sitchensis</i>	Angio	350	700	μmol/mol	738.10	72.92	145.83	4	970.90	118.17	236.34	4	1.32	0.27
Sigurdsson et al. 2001	Gunnesholt, Iceland	WTC	Biomass	g	H-N	854	5.2	63 51 N	-20 13 W	<i>Populus trichocarpa</i>	Angio	Ambient	A+350	μmol/mol	1634.00	146.76	293.52	4	2398.00	240.12	480.24	4	1.47	0.38
Sigurdsson et al. 2001	Gunnesholt, Iceland	WTC	Biomass	g	Low-N	854	5.2	63 51 N	-20 13 W	<i>Populus trichocarpa</i>	Angio	Ambient	A+350	μmol/mol	663.00	72.77	145.53	4	632.00	57.73	115.46	4	0.95	-0.05

References	Site/Location	Type of Experiment	Biomass/ NPP	Parameter Units	Nutrients	Time of Exposure	MAI °C	Lat	Long	Species	Functional Division	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	m2i	Sea	sd2i	n2i	m1i	See	sd1i	n1i	Mean E/A	lnRR
Broadmeadow et al., 2000	Headley, UK	OTC	Biomass	g	None	640	10	52 08 N	-00 50 W	<i>Quercus specios</i>	Angio	365	700	umol/mol	0.20	0.01	0.02	4	0.46	0.05	0.10	4	2.27	0.82
Broadmeadow et al., 2000	Headley, UK	OTC	Biomass	g	None	640	10	52 08 N	-00 50 W	<i>Quercus specios</i>	Angio	365	700	umol/mol	178.42	7.87	15.74	4	314.43	49.47	98.93	4	1.76	0.57
Broadmeadow et al., 2000	Headley, UK	OTC	Biomass	g	None	1037	10	52 08 N	-00 50 W	<i>Fraxinus excelsior</i>	Angio	350	700	umol/mol	0.29	0.02	0.03	2	0.27	0.01	0.01	2	0.92	-0.08
Broadmeadow et al., 2000	Headley, UK	OTC	Biomass	g	None	1037	10	52 08 N	-00 50 W	<i>Quercus petraea</i>	Angio	350	700	umol/mol	0.39	0.01	0.02	2	0.36	0.06	0.06	2	1.05	0.05
Broadmeadow et al., 2000	Headley, UK	OTC	Biomass	g	None	1037	10	52 08 N	-00 50 W	<i>Pinus sylvestris</i>	Gymno	350	700	umol/mol	0.14	0.01	0.01	2	0.21	0.01	0.02	2	1.56	0.44
Reddy et al., 2010	Hyderabad, India	OTC	Biomass	kg	Intmd-N	60	27	17 10 N	78 23 E	<i>Gmelina arborea</i>	Angio	360	460	umol/mol	29.63	0.84	1.67	4	43.64	1.56	3.12	4	1.47	0.39
Day et al., 2013	Merritt, PA, USA	OTC	Biomass	g/m <sup>2</sup>	None	4015	22.4	28 38 N	-80 42 W	<i>Quercus specios</i>	Angio	Ambient	A+350	uL/L	13158.52	460.40	1302.20	8	15749.83	543.44	1537.09	8	1.20	0.18
Norby et al., 2000	ORNL, TN, USA	OTC	Biomass	g	None	180	14.6	35 54 N	-84 20 W	<i>Acer rubrum</i>	Angio	300	600	umol/mol	49.70	15.90	27.54	3	95.70	5.20	9.01	3	1.93	0.66
Norby et al., 2000	ORNL, TN, USA	OTC	Biomass	g	None	180	14.6	35 54 N	-84 20 W	<i>Acer saccharum</i>	Angio	300	600	umol/mol	45.40	4.20	7.27	3	58.20	2.20	3.81	3	1.28	0.25
Norby et al., 2010	ORNL, TN, USA	FACE	NPP	g/m <sup>2</sup> /yr	None	1825	14.6	35 54 N	-84 20 W	<i>Liquidambar styraciflua</i>	Angio	391	542	ppm	1987.27	89.04	154.23	3	2457.69	112.37	158.91	2	1.24	0.21
Norby et al., 1995	ORNL, TN, USA	OTC	Biomass	g	None	4-growing seasons	14.6	35 54 N	-84 20 W	<i>Quercus alba</i>	Angio	Ambient	A+30	Pa	904.00	298.29	667.00	5	1424.00	449.45	1005.00	5	1.58	0.45
Norby et al., 1995	ORNL, TN, USA	OTC	Biomass	g	None	4-growing seasons	14.6	35 54 N	-84 20 W	<i>Quercus alba</i>	Angio	Ambient	A+30	Pa	904.00	298.29	667.00	5	2124.00	588.09	1315.00	5	2.35	0.85
Idso and Kimball, 1994	Phoenix, AR, USA	OTC	Biomass	kg	None	730	21.9	33 26 N	-112 20 W	<i>Pinus edlanca</i>	Gymno	408	554	uL/L	1.18	0.08	0.12	2	2.06	0.32	0.46	2	1.74	0.56
Idso and Kimball, 1994	Phoenix, AR, USA	OTC	Biomass	kg	None	730	21.9	33 26 N	-112 20 W	<i>Pinus edlanca</i>	Gymno	408	680	uL/L	1.18	0.08	0.12	2	3.64	0.48	0.68	2	3.08	1.12
Idso and Kimball, 1994	Phoenix, AR, USA	OTC	Biomass	kg	None	730	21.9	33 26 N	-112 20 W	<i>Pinus edlanca</i>	Gymno	408	812	uL/L	1.18	0.08	0.12	2	4.42	0.22	0.30	2	3.74	1.32
Norby et al., 1992	ORNL, TN, USA	OTC	Biomass	kg	None	2.7 growing seasons	14.6	35 54 N	-84 20 W	<i>Liriodendron tulipifera</i>	Angio	Ambient	A+300	umol/mol	2.17	0.20	0.44	5	2.57	0.30	0.68	5	1.19	0.17
Norby et al., 1992	ORNL, TN, USA	OTC	Biomass	kg	None	2.7 growing seasons	14.6	35 54 N	-84 20 W	<i>Liriodendron tulipifera</i>	Angio	Ambient	A+300	umol/mol	2.17	0.20	0.44	5	2.49	0.21	0.48	5	1.15	0.14
Kimball et al., 2007	Phoenix, AR, USA	OTC	Biomass	kg/tree	High-N	17-years	21.9	33 19 N	-111 48 W	<i>Citrus aurantium</i>	Angio	Ambient	A+300	umol/mol	274.80	9.00	18.00	4	413.80	16.70	33.40	4	1.51	0.41
Johnson et al., 1997	Placerville, CA, USA	OTC	Biomass	g	High-N	3-growing seasons	14.1	38 34 N	-120 45 W	<i>Pinus ponderosa</i>	Gymno	352	700	uL/L	1386.00	136.00	235.56	3	1218.00	85.00	147.22	3	0.88	-0.13
Johnson et al., 1997	Placerville, CA, USA	OTC	Biomass	g	High-N	3-growing seasons	14.1	39 34 N	-120 45 W	<i>Pinus ponderosa</i>	Gymno	352	700	uL/L	1386.00	136.00	235.56	3	2363.00	348.00	602.79	3	1.70	0.53
Johnson et al., 1997	Placerville, CA, USA	OTC	Biomass	g	Low-N	3-growing seasons	14.1	40 34 N	-120 45 W	<i>Pinus ponderosa</i>	Gymno	352	700	uL/L	689.00	76.00	131.64	3	759.00	94.00	162.81	3	1.10	0.10
Johnson et al., 1997	Placerville, CA, USA	OTC	Biomass	g	Low-N	3-growing seasons	14.1	41 34 N	-120 45 W	<i>Pinus ponderosa</i>	Gymno	352	700	uL/L	689.00	76.00	131.64	3	1244.00	191.00	330.82	3	1.81	0.59
Johnson et al., 1997	Placerville, CA, USA	OTC	Biomass	g	Intmd-N	3-growing seasons	14.1	42 34 N	-120 45 W	<i>Pinus ponderosa</i>	Gymno	352	700	uL/L	941.00	121.00	209.58	3	1407.00	174.00	301.38	3	1.50	0.40
King et al., 2005	Rhineland, WI, USA	FACE	NPP	g/m <sup>2</sup>	None	2555	4.3	45 45 N	-89 37.5 E	<i>Aspen mix</i>	Angio	Ambient	560	uL/L	424.27	6.08	10.53	3	541.12	50.02	86.63	3	1.28	0.24
King et al., 2005	Rhineland, WI, USA	FACE	NPP	g/m <sup>2</sup>	None	2555	4.3	46 45 N	-89 37.5 E	<i>Aspen mix</i>	Angio	Ambient	560	uL/L	283.11	10.29	17.82	3	486.33	44.00	76.20	3	1.72	0.54
Rikonen et al., 2004	Suonenjoki, Finland	OTC	Biomass	kg	Intmd-N	3-growing seasons	3.8	62 39 N	27 03 E	<i>Betula pendula</i>	Angio	360	720	ppm	10.38	1.93	3.88	4	10.14	0.93	1.86	4	0.98	-0.02
Rikonen et al., 2004	Suonenjoki, Finland	OTC	Biomass	kg	Intmd-N	3-growing seasons	3.8	62 39 N	27 03 E	<i>Betula pendula</i>	Angio	360	720	ppm	10.56	0.63	1.30	4	14.76	1.92	3.84	4	1.40	0.33
Janssens et al., 2005	UIA, Belgium	OTC	Biomass	g	None	1460	10.8	51 10 N	04 24 E	<i>Pinus sylvestris</i>	Gymno	Ambient	A+400	umol/mol	789.00	97.00	216.90	5	1132.00	211.00	471.81	5	1.43	0.36
Zak et al., 2000	UMBS, MI, USA	OTC	Biomass	kg/chamber	Hi-N	2.5-growing seasons	5.9	45 34 N	-84 40 W	<i>Populus tremuloides</i>	Gymno	36	71	Pa	13.50	0.37	0.84	5	18.61	0.88	1.97	5	1.38	0.32
Zak et al., 2000	UMBS, MI, USA	OTC	Biomass	kg/chamber	Low-N	2.5-growing seasons	5.9	45 34 N	-84 40 W	<i>Populus tremuloides</i>	Gymno	36	71	Pa	4.61	0.36	0.79	5	5.33	0.62	1.38	5	1.16	0.15
Mkan et al., 2000	UMBS, MI, USA	OTC	Biomass	g/m <sup>2</sup>	Hi-N	2-growing seasons	5.9	45 34 N	-84 40 W	<i>Populus tremuloides</i>	Gymno	36	71	Pa	1047.00	43.00	121.62	8	1576.00	104.00	294.18	8	1.51	0.41
Mkan et al., 2000	UMBS, MI, USA	OTC	Biomass	g/m <sup>2</sup>	Low-N	2-growing seasons	5.9	45 34 N	-84 40 W	<i>Populus tremuloides</i>	Gymno	36	71	Pa	485.00	47.00	132.94	8	614.00	51.00	144.25	8	1.27	0.24
Vogel et al., 1997	UMBS, MI, USA	OTC	Biomass	g	None	160	5.9	45 34 N	-84 40 W	<i>Pinus glutinosa</i>	N2-fixing	35	70	Pa	71.70	2.10	4.70	5	110.70	4.38	9.79	5	1.54	0.43
Pregitzer et al., 1995	UMBS, MI, USA	OTC	Biomass	g	Hi-N	158	5.9	45 34 N	-84 40 W	<i>Populus euramericana</i>	Gymno	35	70	Pa	381.60	28.80	64.40	5	562.80	27.90	62.39	5	1.47	0.39
Pregitzer et al., 1995	UMBS, MI, USA	OTC	Biomass	g	Low-N	158	5.9	45 34 N	-84 40 W	<i>Populus euramericana</i>	Gymno	35	70	Pa	298.20	43.10	96.37	5	374.40	49.00	109.57	5	1.26	0.23
Zak et al., 1993	UMBS, MI, USA	OTC	Biomass	g/chamber	None	152	5.9	45 34 N	-84 40 W	<i>Populus grandidentata</i>	Gymno	342	692	umol/mol	71.50	9.90	19.80	4	95.90	8.40	16.80	4	1.34	0.29
Callapetra et al., 2003	Viterbo, Italy	FACE	NPP	mg/ha	None	3-growing seasons	16	42 22 N	11 48 E	<i>Populus euramericana</i>	Gymno	Ambient	550	umol/mol	46.03	4.41	52.92	144	58.50	4.88	58.56	144	1.27	0.24
Callapetra et al., 2003	Viterbo, Italy	FACE	NPP	mg/ha	None	3-growing seasons	16	42 22 N	11 48 E	<i>Populus alba</i>	Gymno	Ambient	550	umol/mol	47.90	6.41	76.92	144	61.84	5.78	69.36	144	1.29	0.26
Callapetra et al., 2003	Viterbo, Italy	FACE	NPP	mg/ha	None	3-growing seasons	16	42 22 N	11 48 E	<i>Populus nigra</i>	Gymno	Ambient	550	umol/mol	62.02	4.57	54.84	144	72.03	4.39	52.68	144	1.16	0.15
Watanabe et al., 2013	Sapporo, Japan	FACE	Biomass	g	None	520days	7.6	43 06 N	141 20 E	<i>Larix gmelinii</i>	Gymno	370	500	umol/mol	236.80	15.53	26.90	3	274.80	12.07	20.90	3	1.16	0.15
Watanabe et al., 2013	Sapporo, Japan	FACE	Biomass	g	None	520days	7.6	43 06 N	141 20 E	<i>Larix gmelinii</i>	Gymno	370	500	umol/mol	233.50	8.66	15.00	3	267.20	24.88	43.10	3	1.14	0.13
Sjurdsson et al., 2013	Flakaliden, Sweden	WTC	Biomass	g/tree	Low-N	1095 days	2	64 07 N	19 17 E	<i>Picea Abies</i>	Gymno	365	700	umol/mol	959.60	70.70	122.46	3	909.09	60.61	104.97	3	0.95	-0.05
Sjurdsson et al., 2013	Flakaliden, Sweden	WTC	Biomass	g/tree	High-N	1095 days	2	64 07 N	19 17 E	<i>Picea Abies</i>	Gymno	365	700	umol/mol	1888.89	383.84	664.83	3	2282.83	191.92	332.42	3	1.21	0.19
Sjurdsson et al., 2013	Flakaliden, Sweden	WTC	Biomass	g/tree	None	1095 days	2	64 07 N	19 17 E	<i>Picea Abies</i>	Gymno	365	700	umol/mol	521.28	25.53	44.22	3	393.62	31.92	55.28	3	0.76	-0.28
Barton et al., 2011	Richmond, Australia	WTC	Biomass	g/tree	None	5 years	17	33 36 S	150 44 E	<i>Eucalyptus saligna</i>	Angio	Ambient	A+240	ppm	24890.87	2261.37	3916.80	3	13846.73	3095.10	5360.86	3	0.56	-0.59
Dawes et al., 2011	Davos, Switzerland	FACE	Shoot growth	mm	None	9 years	1.8	46 46 N	09 52 E	<i>Larix decidua</i>	Gymno	Ambient	550	umol/mol	17.22	3.33	10.54	10	27.78	4.17	13.18	10	1.61	0.48
Dawes et al., 2011	Davos, Switzerland	FACE	Shoot growth	mm	None	9 years	1.8	46 46 N	09 52 E	<i>Pinus mugo</i>	Gymno	Ambient	550	umol/mol	4.03	0.83	2.64	10	4.86	0.56	1.78	10	1.21	0.19
Paltola et al., 2002	Mekrijarvi, Finland	CTC	Biomass	g/m <sup>2</sup>	None	3-growing seasons	2.5	62 47 N	30 58 E	<i>Pinus sylvestris</i>	Gymno	Ambient	700	umol/mol	12.28	4.74	9.48	4	29.91	0.36	0.72	4	2.44	0.89
Lovelock et al., 1998	Parque Natural Metropolitano, Panama.	OTC	Biomass	g	None	6 months	26.3	08 59 N	79 33 W	<i>Tree communities</i>	Angio	350	700	ppm	1502.00	174.00	301.38	3	1406.00	182.00	315.23	3	0.94	-0.07
Ceulemans et al., 1996	UIA, Belgium	OTC	Biomass	g	High-N	600	10.8	51 10 N	04 24 E	<i>Poplar Beaupre</i>	Angio	Ambient	A+350	umol/mol	0.20				0.34				1.68	0.52
Ceulemans et al., 1996	UIA, Belgium	OTC	Biomass	g	High-N	600	10.8	51 10 N	04 24 E	<i>Poplar Robusta</i>	Angio	Ambient	A+350	umol/mol	0.25				0.40				1.62	0.48
Laitat et al., 1994	Vielsalm, Belgium	OTC	Biomass	g	Low-N	1280	7.5	50 17 N	05 55 E	<i>Picea abies</i>	Gymno	350	700	umol/mol	0.08			2	0.08			2	1.05	0.05
Badeck et al., 1997	Université de Paris-Sud, France	Mini-ecosystem	Biomass	g	High-N	3-growing seasons	15	48 42 N	2 09 E	<i>Fagus sylvatica</i>	Angio	350	700	umol/mol										

## Abbreviations used in Table A1 & A2:

Code	Abbreviations	Code	Abbreviations
ABGRWT	Above-ground Weight	LCLTSd	Low CO <sub>2</sub> Low Temperature Standard deviation
CO <sub>2</sub> inc	CO <sub>2</sub> increment	LCLTSe	Low CO <sub>2</sub> Low Temperature Standard error
CTC	Closed Top Chambers	lnRR	Log Response Ratio
FACE	Free-air-CO <sub>2</sub> -enrichment	low CI	Low er Confidence Interval
GC	Growth Chambers	m1i	High CO <sub>2</sub> mean
GH	Green/ Glass House	m2i	Low CO <sub>2</sub> mean
HCHT	High CO <sub>2</sub> High Temperature Mean	n1i	High CO <sub>2</sub> No. of replicates
HCHTN	High CO <sub>2</sub> High Temperature No. of replicates	n2i	Low CO <sub>2</sub> No. of replicates
HCHTSd	High CO <sub>2</sub> High Temperature Standard deviation	NA	Not Applicable
HCHTSe	High CO <sub>2</sub> High Temperature Standard error	OTC	Open Top Chambers
HCLT	High CO <sub>2</sub> Low Temperature Mean	RTWT	Root Weight
HCLTN	High CO <sub>2</sub> Low Temperature No. of replicates	sd1i	High CO <sub>2</sub> Standard deviation
HCLTSd	High CO <sub>2</sub> Low Temperature Standard deviation	sd2i	Low CO <sub>2</sub> Standard deviation
HCLTSe	High CO <sub>2</sub> Low Temperature Standard error	Se1i	High CO <sub>2</sub> Standard error
hiCI	Higher Confidence Interval	Se2i	Low CO <sub>2</sub> Standard error
LCHT	Low CO <sub>2</sub> High Temperature Mean	STWT	Stem Weight
LCHTN	Low CO <sub>2</sub> High Temperature No. of replicates	TW	Total weight
LCHTSd	Low CO <sub>2</sub> High Temperature Standard deviation	Var	Variance
LCHTSe	Low CO <sub>2</sub> High Temperature Standard error	W	Weight
LCLT	Low CO <sub>2</sub> Low Temperature Mean	WTC	Whole Tree Chambers
LCLTN	Low CO <sub>2</sub> Low Temperature No. of replicates		

## References (Table A1):

References	
Bauweraerts et al., 2013	Bauweraerts, I., T. M. Werten, M. Amey, M. A. McGuire, R. O. Teskey, and K. Steppe. 2013. The effect of heat waves, elevated [CO <sub>2</sub> ] and low soil water availability on northern red oak ( <i>Quercus rubra</i> L.) seedlings. <i>Global Change Biology</i> 19:517-528.
Bruhn et al., 2000	Bruhn, D., J. W. Leverenz, and H. Saxe. 2000. Effects of tree size and temperature on relative growth rate and its components of <i>Fagus sylvatica</i> seedlings exposed to two partial pressures of atmospheric [CO <sub>2</sub> ]. <i>New Phytologist</i> 146:415-425.
Callaway et al., 1994	Callaway, R. M., E. H. Delucia, E. M. Thomas, and W. H. Schlesinger. 1994. Compensatory Responses of CO <sub>2</sub> Exchange and Biomass Allocation and Their Effects on the Relative Growth-Rate of Ponderosa Pine in Different CO <sub>2</sub> and Temperature Regimes. <i>Oecologia</i> 98:159-166.
Delucia et al., 1997	Delucia, E. H., R. M. Callaway, E. M. Thomas, and W. H. Schlesinger. 1997. Mechanisms of phosphorus acquisition for ponderosa pine seedlings under high CO <sub>2</sub> and temperature. <i>Annals of Botany</i> 79:111-120.
Duan et al., 2013	Duan, H., J. S. Amthor, R. A. Duursma, A. P. O'Grady, B. Choat, and D. T. Tissue. 2013. Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO <sub>2</sub> ] and elevated temperature. <i>Tree Physiology</i> 33:779-792.
Ghannoum et al., 2010	Ghannoum, O., N. G. Phillips, J. P. Conroy, R. A. Smith, R. D. Attard, R. Woodfield, B. A. Logan, J. D. Lewis, and D. T. Tissue. 2010. Exposure to preindustrial, current and future atmospheric CO <sub>2</sub> and temperature differentially affects growth and photosynthesis in Eucalyptus. <i>Global Change Biology</i> 16:303-319.
Hou et al., 2010	Hou, Y., Z. K. Luo, G. D. Jenerette, Y. Z. Qiao, and K. Y. Wang. 2010. Effects of Elevated CO <sub>2</sub> and Temperature on Growth and Morphology of Fir ( <i>Abies Faxoniana</i> Rehd. <i>Et Wils.</i> ) and Native Herbs in a Treeline Ecotone: An Experimental Approach. <i>Polish Journal of Ecology</i> 58:311-322.
Kellomaki and Wang, 2001	Kellomaki, S. and K. Y. Wang. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. <i>Annals of Botany</i> 87:669-682.
King et al., 1996	King, J. S., R. B. Thomas, and B. R. Strain. 1996. Growth and carbon accumulation in root systems of Pinus taeda and Pinus ponderosa seedlings as affected by varying CO <sub>2</sub> , temperature and nitrogen. <i>Tree Physiology</i> 16:635-642.
Kuokkanen et al., 2001	Kuokkanen, K., R. Julkunen-Tiitto, M. Keinänen, P. Niemela, and J. Tahvanainen. 2001. The effect of elevated CO <sub>2</sub> and temperature on the secondary chemistry of <i>Betula pendula</i> seedlings. <i>Trees-Structure and Function</i> 15:378-384.
Lavola et al., 2013	Lavola, A., L. Nybakken, M. Rousi, J. Pusenius, M. Petrelius, S. Kellomaki, and R. Julkunen-Tiitto. 2013. Combination treatment of elevated UVB radiation, CO <sub>2</sub> and temperature has little effect on silver birch ( <i>Betula pendula</i> ) growth and phytochemistry. <i>Physiologia Plantarum</i> 149:499-514.
Lewis et al., 2013	Lewis, J. D., R. A. Smith, O. Ghannoum, B. A. Logan, N. G. Phillips, and D. T. Tissue. 2013. Industrial-age changes in atmospheric [CO <sub>2</sub> ] and temperature differentially alter responses of faster- and slower-growing Eucalyptus seedlings to short-term

References	
	drought. Tree Physiology 33:475-488.
Maherali and DeLucia, 2000	Maherali, H. and E. H. DeLucia. 2000. Interactive effects of elevated CO <sub>2</sub> and temperature on water transport in ponderosa pine. American Journal of Botany 87:243-249.
Mortenson, 1996	Mortensen, L. M. 1995. Effect of Carbon-Dioxide Concentration on Biomass Production and Partitioning in <i>Betula-Pubescent</i> Ehrh Seedlings at Different Ozone and Temperature Regimes. Environmental Pollution 87:337-343.
Norby and Luo, 2004	Norby, R. J. and Y. Q. Luo. 2004. Evaluating ecosystem responses to rising atmospheric CO <sub>2</sub> and global warming in a multi-factor world. New Phytologist 162:281-293.
Olszyk et al., 2003	Olszyk, D. M., M. G. Johnson, D. T. Tingey, P. T. Rygielwicz, C. Wise, E. VanEss, A. Benson, M. J. Storm, and R. King. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO <sub>2</sub> and temperature for 4 years. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 33:269-278.
Overdieck et al., 2007	Overdieck, D., D. Ziche, and K. Bottcher-Jungdhaus. 2007. Temperature responses of growth and wood anatomy in European beech saplings grown in different carbon dioxide concentrations. Tree Physiology 27:261-268.
Sallas et al., 2003	Sallas, L., E. M. Luomala, J. Utriainen, P. Kainulainen, and J. K. Holopainen. 2003. Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. Tree Physiology 23:97-108.
Sheu and Lin, 1999	Sheu, B. H. and C. K. Lin. 1999. Photosynthetic response of seedlings of the sub-tropical tree <i>Schima superba</i> with exposure to elevated carbon dioxide and temperature. Environmental and Experimental Botany 41:57-65.
Sigurdsson et al., 2013	Sigurdsson, B. D., J. L. Medhurst, G. Wallin, O. Eggertsson, and S. Linder. 2013. Growth of mature boreal Norway spruce was not affected by elevated [CO <sub>2</sub> ] and/or air temperature unless nutrient availability was improved. Tree Physiology 33:1192-1205.
Tjoelker et al., 1998	Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1998. Temperature and ontogeny mediate growth response to elevated CO <sub>2</sub> in seedlings of five boreal tree species. New Phytologist 140:197-210.
Usami et al., 2001	Usami, T., J. Lee, and T. Oikawa. 2001. Interactive effects of increased temperature and CO <sub>2</sub> on the growth of <i>Quercus myrsinaefolia</i> saplings. Plant Cell and Environment 24:1007-1019.
Uselman et al., 2000	Uselman, S. M., R. G. Qualls, and R. B. Thomas. 2000. Effects of increased atmospheric CO <sub>2</sub> , temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree ( <i>Robinia pseudoacacia</i> L.). Plant and Soil 222:191-202.
Veteli et al., 2002	Veteli, T. O., K. Kuokkanen, R. Julkunen-Tiitto, H. Roininen, and J. Tahvanainen. 2002. Effects of elevated CO <sub>2</sub> and temperature on plant growth and herbivore defensive chemistry. Global Change Biology 8:1240-1252.
Wan et al., 2004	Wan, S. Q., R. J. Norby, K. S. Pregitzer, J. Ledford, and E. G. O'Neill. 2004. CO <sub>2</sub> enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. New Phytologist 162:437-446.
Wayne et al., 1998	Wayne, P. M., E. G. Reekie, and F. A. Bazzaz. 1998. Elevated CO <sub>2</sub> ameliorates birch response to high temperature and frost stress: implications for modeling climate-induced geographic range shifts. Oecologia 114:335-342.

## References (Table A2):

References	
Badeck et al., 1997	Badeck F.-W., D. E., Epron D., Le Dantec V., Liozon R., Mousseau M., Pontallier J.-Y. and Saugier B. 1997. Sweet chestnut and beech saplings under elevated CO <sub>2</sub> . Pages 15-25. in K. K. a. S. S. G. M. J. Mohren, editor. Impacts of Global Change on Tree Physiology and Forest Ecosystems. Kluwer Academic Publishers, Dordrecht.
Barker et al., 2005	Barker, D. H., B. R. Loveys, J. J. G. Egerton, H. Gorton, W. E. Williams, and M. C. Ball. 2005. CO <sub>2</sub> enrichment predisposes foliage of a eucalypt to freezing injury and reduces spring growth. Plant Cell and Environment 28:1506-1515.
Barton et al., 2011	Barton, C. V. M., R. A. Duursma, B. E. Medlyn, D. S. Ellsworth, D. Eamus, D. T. Tissue, M. A. Adams, J. Conroy, K. Y. Crous, M. Liberloo, M. Low, S. Linder, and R. E. McMurtrie. 2012. Effects of elevated atmospheric [CO <sub>2</sub> ] on instantaneous transpiration efficiency at leaf and canopy scales in Eucalyptus saligna. Global Change Biology 18:585-595.
Broadmeadow et al., 2000	Broadmeadow, M. S. J. and S. B. Jackson. 2000. Growth responses of <i>Quercus petraea</i> , <i>Fraxinus excelsior</i> and <i>Pinus sylvestris</i> to elevated carbon dioxide, ozone and water supply. New Phytologist 146:437-451.
Calfapietra et al., 2003	Calfapietra, C., B. Gielen, A. N. J. Galema, M. Lukac, P. De Angelis, M. C. Moscatelli, R. Ceulemans, and G. Scarascia-Mugnozza. 2003. Free-air CO <sub>2</sub> enrichment (FACE) enhances biomass production in a short-rotation poplar plantation. Tree Physiology 23:805-814.
Ceulemans et al., 1996	Ceulemans, R., B. Y. Shao, X. N. Jiang, and J. Kalina. 1996. First- and second-year aboveground growth and productivity of two Populus hybrids grown at ambient and elevated CO <sub>2</sub> . Tree Physiology 16:61-68.
Dawes et al., 2011	Dawes, M. A., S. Hattenschwiler, P. Bebi, F. Hagedorn, I. T. Handa, C. Körner, and C. Rixen. 2011. Species-specific tree growth responses to 9 years of CO <sub>2</sub> enrichment at the alpine treeline. Journal of Ecology 99:383-394.
Day et al., 2013	Day, F. P., R. E. Schroeder, D. B. Stover, A. L. P. Brown, J. R. Butnor, J. Dilustro, B. A. Hungate, P. Dijkstra, B. D. Duval, T. J. Seiler, B. G. Drake, and C. R. Hinkle. 2013. The effects of 11 yr of CO <sub>2</sub> enrichment on roots in a Florida scrub-oak ecosystem. New Phytologist 200:778-787.
Forstreuter 1995	ECOCRAFT. 1999. Predicted impacts of rising carbon dioxide and temperature on forests in Europe at stand scale. Institute of Ecology and Resource Management, University of Edinburgh, Edinburgh, UK.
Goodfellow et al., 1997	Goodfellow, J., D. Eamus, and G. Duff. 1997. Diurnal and seasonal changes in the impact of CO <sub>2</sub> enrichment on assimilation, stomatal conductance and growth in a long-term study of <i>Mangifera indica</i> in the wet-dry tropics of Australia. Tree Physiology 17:291-299.
Idso and Kimball, 1994	Idso, S. B. and B. A. Kimball. 1994. Effects of Atmospheric CO <sub>2</sub> Enrichment on Biomass Accumulation and Distribution in Eldarica Pine Trees. Journal of Experimental Botany 45:1669-1672.
Janssens et al., 2005	Janssens, I. A., B. Medlyn, B. Gielen, I. Laureysens, M. E. Jach, D. Van Hove, and R. Ceulemans. 2005. Carbon budget of Pinus sylvestris saplings after four years of exposure to elevated atmospheric carbon dioxide concentration. Tree Physiology 25:325-337.
Johnson et al., 1997	Johnson, D. W., J. T. Ball, and R. F. Walker. 1997. Effects of CO <sub>2</sub> and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. Plant and Soil 190:29-40.
Kimball et al., 2007	Kimball, B. A., S. B. Idso, S. Johnson, and M. C. Rillig. 2007. Seventeen years of carbon dioxide enrichment of sour orange trees: final results. Global Change Biology 13:2171-2183.
King et al., 2005	King, J. S., M. E. Kubiske, K. S. Pregitzer, G. R. Hendrey, E. P. McDonald, C. P. Giardina, V. S. Quinn, and D. F. Karnosky. 2005. Tropospheric CO <sub>2</sub> compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO <sub>2</sub> . New Phytologist 168:623-635.

References	
Laitat et al., 1994	Laitat, E., Loosveldt, P., Boussard, H. and Hirvijarvi, E. 1994. Study on major morphological, physiological and biochemical processes likely to be affected under combined effects of increasing atmospheric CO <sub>2</sub> concentrations and elevated temperature in partial ecosystem enclosures. Pages 37-52. in R. C. F. Veroustraete, I. Impens and J. Van Rensbergen, editor. Vegetation, Modelling and Climate Change Effects. SPB Academic Publishing, The Hague, The Netherlands.
Laitat et al., 1999	Laitat, E., R. Ceulemans, B. E. Medlyn, F. LeFevre, M. Forstreuter, E. Jach, A. Rey, J. Strassmeyer, and P. G. Jarvis. 1999. Carbon uptake and allocation. Predicted Impacts of Rising Carbon Dioxide and Temperature on Forests in Europe at Stand Scale. Final Report, ECOCRAFT Project, University of Edinburgh, UK.
Lovelock et al., 1998	Lovelock, C. E., K. Winter, R. Mersits, and M. Popp. 1998. Responses of communities of tropical tree species to elevated CO <sub>2</sub> in a forest clearing. <i>Oecologia</i> 116:207-218.
Loveys et al 2010	Loveys, B. R., J. J. G. Egerton, D. Bruhn, and M. C. Ball. 2010. Disturbance is required for CO <sub>2</sub> -dependent promotion of woody plant growth in grasslands. <i>Functional Plant Biology</i> 37:555-565.
McCarthy et al, 2010	McCarthy, H. R., R. Oren, K. H. Johnsen, A. Gallet-Budynek, S. G. Pritchard, C. W. Cook, S. L. LaDeau, R. B. Jackson, and A. C. Finzi. 2010. Re-assessment of plant carbon dynamics at the Duke free-air CO <sub>2</sub> enrichment site: interactions of atmospheric [CO <sub>2</sub> ] with nitrogen and water availability over stand development. <i>New Phytologist</i> 185:514-528.
Mikan et al., 2000	Mikan, C. J., D. R. Zak, M. E. Kubiske, and K. S. Pregitzer. 2000. Combined effects of atmospheric CO <sub>2</sub> and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. <i>Oecologia</i> 124:432-445.
Norby et al., 1992	Norby, R. J., C. A. Gunderson, S. D. Wullschlegel, E. G. O'Neill, and M. K. McCracken. 1992. Productivity and Compensatory Responses of Yellow-Poplar Trees in Elevated CO <sub>2</sub> . <i>Nature</i> 357:322-324.
Norby et al., 1995	Norby, R. J., S. D. Wullschlegel, C. A. Gunderson, and C. T. Nitch. 1995. Increased Growth Efficiency of <i>Quercus-Alba</i> Trees in a CO <sub>2</sub> -Enriched Atmosphere. <i>New Phytologist</i> 131:91-97.
Norby et al., 2000	Norby, R. J., T. M. Long, J. S. Hartz-Rubin, and E. G. O'Neill. 2000. Nitrogen resorption in senescing tree leaves in a warmer, CO <sub>2</sub> -enriched atmosphere. <i>Plant and Soil</i> 224:15-29.
Norby et al., 2010	Norby, R. J., J. M. Warren, C. M. Iversen, B. E. Medlyn, and R. E. McMurtrie. 2010. CO <sub>2</sub> enhancement of forest productivity constrained by limited nitrogen availability. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 107:19368-19373.
Paltola et al, 2002	Peltola, H., A. Kilpelainen, and S. Kellomaki. 2002. Diameter growth of Scots pine ( <i>Pinus sylvestris</i> ) trees grown at elevated temperature and carbon dioxide concentration under boreal conditions. <i>Tree Physiology</i> 22:963-972.
Pregitzer et al., 1995	Pregitzer, K. S., D. R. Zak, P. S. Curtis, M. E. Kubiske, J. A. Teeri, and C. S. Vogel. 1995. Atmospheric CO <sub>2</sub> , Soil-Nitrogen and Turnover of Fine Roots. <i>New Phytologist</i> 129:579-585.
Reddy et al., 2010	Reddy, A. R., G. K. Rasineni, and A. S. Raghavendra. 2010. The impact of global elevated CO <sub>2</sub> concentration on photosynthesis and plant productivity. <i>Current Science</i> 99:46-57.
Rey and Jarvis, 1997	REY, A. and P. G. JARVIS. 1997. Growth Response of Young Birch Trees ( <i>Betula pendula</i> Roth.) After Four and a Half Years of CO <sub>2</sub> Exposure. <i>Annals of Botany</i> 80:809-816.
Riikonen et al., 2004	Riikonen, J., M. M. Lindsberg, T. Holopainen, E. Oksanen, J. Lappi, P. Peltonen, and E. Vapaavuori. 2004. Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. <i>Tree Physiology</i> 24:1227-1237.
Roden et al., 1999	Roden, J. S., J. J. G. Egerton, and M. C. Ball. 1999. Effect of elevated [CO <sub>2</sub> ] on photosynthesis and growth of snow gum ( <i>Eucalyptus pauciflora</i> ) seedlings during winter and spring. <i>Australian Journal of Plant Physiology</i> 26:37-46.
Sigurdsson et al, 2001	Sigurdsson, B. D., H. Thorgeirsson, and S. Linder. 2001. Growth and dry-matter partitioning of young <i>Populus trichocarpa</i> in response to carbon dioxide concentration and mineral nutrient availability. <i>Tree Physiology</i> 21:941-950.
Sigurdsson et al., 2013	Sigurdsson, B. D., J. L. Medhurst, G. Wallin, O. Eggertsson, and S. Linder. 2013. Growth of mature boreal Norway spruce was not affected by elevated [CO <sub>2</sub> ] and/or air temperature unless nutrient availability was improved. <i>Tree Physiology</i> 33:1192-1205.
Smith et al., 2013	Smith, A. R., M. Lukac, R. Hood, J. R. Healey, F. Miglietta, and D. L. Godbold. 2013. Elevated CO <sub>2</sub> enrichment induces a differential biomass response in a mixed species temperate forest plantation. <i>New Phytologist</i> 198:156-168.
Spinnler et al., 2002	Spinnler, D., P. Egh, and C. Körner. 2002. Four-year growth dynamics of beech-spruce model ecosystems under CO <sub>2</sub> enrichment on two different forest soils. <i>Trees-Structure and Function</i> 16:423-436.
Temperton et al., 2003	Temperton, V. M., S. J. Grayston, G. Jackson, C. V. M. Barton, P. Millard, and P. G. Jarvis. 2003. Effects of elevated carbon dioxide concentration on growth and nitrogen fixation in <i>Alnus glutinosa</i> in a long-term field experiment. <i>Tree Physiology</i> 23:1051-1059.
Tissue et al., 1997	Tissue, D. T., R. B. Thomas, and B. R. Strain. 1997. Atmospheric CO <sub>2</sub> enrichment increases growth and photosynthesis of <i>Pinus taeda</i> : a 4 year experiment in the field. <i>Plant Cell and Environment</i> 20:1123-1134.
Vogel et al., 1997	Vogel, C. S., P. S. Curtis, and R. B. Thomas. 1997. Growth and nitrogen accretion of dinitrogen-fixing <i>Alnus glutinosa</i> (L) Gaertn under elevated carbon dioxide. <i>Plant Ecology</i> 130:63-70.
Watanabe et al., 2013	Watanabe, M., Q. Mao, E. Novrianti, K. Kita, K. Takagi, F. Satoh, and T. Koike. 2013. Elevated CO <sub>2</sub> enhances the growth of hybrid larch F1 ( <i>Larix gmelinii</i> var. <i>japonica</i> × <i>L. kaempferi</i> ) seedlings and changes its biomass allocation. <i>Trees</i> 27:1647-1655.
Zak et al., 1993	Zak, D. R., K. S. Pregitzer, P. S. Curtis, J. A. Teeri, R. Fogel, and D. L. Randlett. 1993. Elevated Atmospheric CO <sub>2</sub> and Feedback between Carbon and Nitrogen Cycles. <i>Plant and Soil</i> 151:105-117.
Zak et al., 2000	Zak, D. R., K. S. Pregitzer, P. S. Curtis, C. S. Vogel, W. E. Holmes, and J. Lussenhop. 2000. Atmospheric CO <sub>2</sub> , soil-N availability, and allocation of biomass and nitrogen by <i>Populus tremula</i> L. <i>Ecological Applications</i> 10:34-46.

Appendix B:

Table B1: A list of experiments giving mean values, standard deviations and number of replicates for ambient CO<sub>2</sub> and elevated CO<sub>2</sub> treatments for iWUE, iTE and WUE. For abbreviations refer to abbreviation list at the end of the table B2.

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XSE	XNE	XSA/XNE	Parameter Y	Units Y	YA	YSA	YNE	YSE	YN	Parameter Z	Units Z	ZA	ZSA	ZNE	ZSE	ZN		
Wayne et al., 1997	MA, USA	GC	WUE	mg/gH <sub>2</sub> O	61 days	<i>Betula alleghaniensis</i>	C3	Woody	Angio	400	800	u/l	100	Temperature		18.925	12.18	28.18	18.00	Biomass	g	4.07	1.10	18	6.36	2.25	18	Transpiration rate	gH <sub>2</sub> O/cm <sup>2</sup> /d	0.12	0.02	18	0.09	0.02	18		
Wayne et al., 1997	MA, USA	GC	WUE	mg/gH <sub>2</sub> O	61 days	<i>Betula alleghaniensis</i>	C3	Woody	Angio	400	800	u/l	100	Temperature		17.83	2.80	18	25.73	3.48	18.00	Biomass	g	1.10	0.55	18	3.66	1.27	18	Transpiration rate	gH <sub>2</sub> O/cm <sup>2</sup> /d	0.21	0.02	18	0.14	0.02	18
Werten et al., 2010.	GA, USA	GC	WUE	umolCO <sub>2</sub> /mmolH <sub>2</sub> O	133 days	<i>Pinus taeda</i>	C3	Woody	Gymno	380	700	umol/mol	84	H <sub>2</sub> O	Temperature	2.57	0.724	3.39	0.684	0.00	Net Photo	umol/m <sup>2</sup> /s	6.12	0.504	9.25	0.744		St Cond	mmol/m <sup>2</sup> /s	0.16	0.03	4	0.16	0.26	4		
Werten et al., 2010.	GA, USA	GC	WUE	umolCO <sub>2</sub> /mmolH <sub>2</sub> O	133 days	<i>Pinus taeda</i>	C3	Woody	Gymno	350	550	umol/mol	67	H <sub>2</sub> O	Temperature	1.47	0.224	3.32	0.784	0.00	Net Photo	umol/m <sup>2</sup> /s	5.78	0.784	9.67	0.864		St Cond	mmol/m <sup>2</sup> /s	0.19	0.04	4	0.17	0.03	4		
Koike et al., 1996	Japan	GC	iTE	umol/mmol	65 days	<i>Betula platyphylla</i>	C3	Woody	Angio	36	70	Pa	94	Temperature		1.64	0.084	2.04	0.284	0.00																	
Koike et al., 1996	Japan	GC	iTE	umol/mmol	65 days	<i>Betula platyphylla</i>	C3	Woody	Angio	36	70	Pa	94	Temperature		1.38	0.184	1.72	0.184	0.00																	
Koike et al., 1996	Japan	GC	iTE	umol/mmol	65 days	<i>Betula platyphylla</i>	C3	Woody	Angio	36	70	Pa	94	Temperature		1.76	0.204	1.80	0.084	0.00																	
Koike et al., 1996	Japan	GC	iTE	umol/mmol	65 days	<i>Betula platyphylla</i>	C3	Woody	Angio	36	70	Pa	94	Temperature		1.62	0.144	1.72	0.084	0.00																	
Vu et al., 2002	FL, USA	TGG	iTE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	880	<i>Citrus reticulata/paradisi</i>	C3	Woody	Angio	360	720	umol/mol	100	Temperature		2.10	0.204	4.30	0.404	0.00	Net Photo	umol/m <sup>2</sup> /s	8.00	0.604	11.60	1.404		Transpiration	mmol/m <sup>2</sup> /s	3.90	0.40	4	2.70	0.40	4		
Vu et al., 2002	FL, USA	TGG	iTE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	880	<i>Citrus reticulata/paradisi</i>	C3	Woody	Angio	360	720	umol/mol	100	Temperature		1.60	0.204	4.00	0.404	0.00	Net Photo	umol/m <sup>2</sup> /s	9.10	0.604	12.90	1.204		Transpiration	mmol/m <sup>2</sup> /s	5.70	1.20	4	3.20	0.60	4		
Ghannoum et al., 2010	Australia	GH	iTE	mmol/mol	150	<i>Eucalyptus saligna</i>	C3	Woody	Angio	400	650	u/L	62	Temperature		3.09	0.485	5.40	0.865	0.00	Net Photo	umol/m <sup>2</sup> /s	13.41	1.095	23.41	2.735											
Ghannoum et al., 2010	Australia	GH	iTE	mmol/mol	150	<i>Eucalyptus saligna</i>	C3	Woody	Angio	400	650	u/L	62	Temperature		2.57	0.385	3.64	0.985	0.00	Net Photo	umol/m <sup>2</sup> /s	15.34	2.735	23.41	2.735											
Ghannoum et al., 2010	Australia	GH	iTE	mmol/mol	150	<i>Eucalyptus sideroxylon</i>	C3	Woody	Angio	400	650	u/L	62	Temperature		3.60	0.945	5.14	1.085	0.00	Net Photo	umol/m <sup>2</sup> /s	18.23	2.75	27.32	3.85											
Ghannoum et al., 2010	Australia	GH	iTE	mmol/mol	150	<i>Eucalyptus sideroxylon</i>	C3	Woody	Angio	400	650	u/L	62	Temperature		2.53	0.865	3.39	0.775	0.00	Net Photo	umol/m <sup>2</sup> /s	22.44	9.15	29.57	6.35											
Anderson et al./ 1998	WI, USA	GC	iTE	mol/mol	11 weeks	<i>Quercus rubra</i>	C3	Woody	Angio	400	700	umol/mol	75	H <sub>2</sub> O		5.33	2.376	10.52	2.376	0.00	Net Photo	umol/m <sup>2</sup> /s	4.41	1.886	6.40	1.886											
Anderson et al./ 1998	WI, USA	GC	iTE	mol/mol	11 weeks	<i>Quercus rubra</i>	C3	Woody	Angio	400	530	umol/mol	82	H <sub>2</sub> O		5.33	2.376	8.62	2.086	0.00	Net Photo	umol/m <sup>2</sup> /s	4.41	1.886	6.59	1.936											
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora apiculata</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		4.21	0.585	4.80	0.725	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora apiculata</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		1.95	0.835	3.26	0.385	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora apiculata</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		4.68	1.195	4.90	2.885	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora apiculata</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		1.12	0.545	1.91	3.085	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora stylosa</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		3.81	0.945	5.80	0.945	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora stylosa</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		2.35	0.835	3.82	0.475	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora stylosa</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		4.94	2.385	6.50	1.235	0.00																	
Bail et al., 1997	Australia	GH	WUE	g DW/ Kg H <sub>2</sub> O loss	14 weeks	<i>Rhizophora stylosa</i>	C3	Woody	Angio	340	700	cm <sup>3</sup> /m <sup>3</sup>	105	NaCl		2.84	0.425	4.94	2.385	0.00																	
Biswas et al., 2013	China	OTC	WUE	umol/mol	21 days	<i>Triticum aestivum</i>	C4	Crop	Angio	385	714	ppm	85			43.30	12.48	44.03	12.18	0.00	Net Photo	umol/m <sup>2</sup> /s	8.68	1.788	8.31	1.708		St cond	mol/m <sup>2</sup> /s	0.20	0.06	8	0.19	0.06	8		
Biswas et al., 2013	China	OTC	WUE	umol/mol	21 days	<i>Triticum aestivum</i>	C4	Crop	Angio	385	714	ppm	85			43.30	12.38	40.68	12.48	0.00	Net Photo	umol/m <sup>2</sup> /s	8.93	2.128	10.08	2.068		St cond	mol/m <sup>2</sup> /s	0.21	0.06	8	0.25	0.06	8		
Biswas et al., 2013	China	OTC	WUE	umol/mol	21 days	<i>Triticum aestivum</i>	C4	Crop	Angio	385	714	ppm	85	O3		31.11	10.08	51.24	13.68	0.00	Net Photo	umol/m <sup>2</sup> /s	3.75	1.368	8.55	1.478		St cond	mol/m <sup>2</sup> /s	0.13	0.06	8	0.17	0.06	8		
Biswas et al., 2013	China	OTC	WUE	umol/mol	21 days	<i>Triticum aestivum</i>	C4	Crop	Angio	385	714	ppm	85	O3		36.21	12.48	40.72	12.48	0.00	Net Photo	umol/m <sup>2</sup> /s	4.87	1.508	10.08	1.708		St cond	mol/m <sup>2</sup> /s	0.16	0.06	8	0.25	0.06	8		



References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XEA	XNE	Parameter Y	Units Y	YA	YSA	YNA	YEA	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZSE	ZNE		
Cao et al., 2007	Canada	GH	ITE	μmol/mmol	80 days	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	μmol/mol	100	Nitrogen			0.5	1.51	0.35	4.22	1.01	0.0	Net Photo	μmol/m <sup>2</sup> /s	5.37	1.42	10.6	2.0	0.38	Transpiration	mmol/m <sup>2</sup> /s	3.60	0.79	10.1	1.55	0.44	10.1
Cao et al., 2007	Canada	GH	ITE	μmol/mmol	80 days	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	μmol/mol	100	Nitrogen			0.5	2.32	0.54	5.57	0.89	0.0	Net Photo	μmol/m <sup>2</sup> /s	6.89	1.01	10.9	8.7	2.37	Transpiration	mmol/m <sup>2</sup> /s	3.01	0.62	10.1	1.83	0.51	10.1
Cao et al., 2007	Canada	GH	ITE	μmol/mmol	80 days	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	μmol/mol	100	Nitrogen			0.5	2.92	0.51	7.57	1.38	0.0	Net Photo	μmol/m <sup>2</sup> /s	9.80	1.42	10.1	12.0	2.97	Transpiration	mmol/m <sup>2</sup> /s	3.40	0.63	10.1	1.63	0.35	10.1
Cao et al., 2007	Canada	GH	ITE	μmol/mmol	80 days	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	μmol/mol	100	Nitrogen			0.5	2.43	0.70	7.30	1.52	0.0	Net Photo	μmol/m <sup>2</sup> /s	8.98	0.79	10.1	13.22	1.99	Transpiration	mmol/m <sup>2</sup> /s	3.71	0.60	10.1	1.88	0.44	10.1
Cao et al., 2007	Canada	GH	ITE	μmol/mmol	80 days	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	μmol/mol	100	Nitrogen			0.5	2.70	0.70	7.51	0.89	0.0	Net Photo	μmol/m <sup>2</sup> /s	8.03	1.00	10.1	11.42	2.80	Transpiration	mmol/m <sup>2</sup> /s	3.03	0.63	10.1	1.52	0.44	10.1
Centritto et al., 1999	UK	OTC	WUE	g/Kg	2 growing seasons	<i>Prunus avium</i>	C3	Woody	Angio	350	700	μmol/mol	100	H <sub>2</sub> O			7.72	0.54	11.39	1.09	0.0	Biomass	g	250.9	40.6	9	346.0	56.3	Water loss	kg	18.1	1.35	6	17.1	1.13	6	
Fredeen et al., 1998	CA, USA	OTM	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	3 growing seasons	<i>Avena fatua</i>	C4	Herbaceous	Angio	A	36	Pa	94			1	11.1	1.90	13.09	2.06	0.0	Net Photo	μmol/m <sup>2</sup> /s	6.93	1.04	6	10.18	1.68									
Fredeen et al., 1998	CA, USA	OTM	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	3 growing seasons	<i>Plantago erecta</i>	C4	Herbaceous	Angio	A	36	Pa	94			1	9.83	3.58	14.7	4.37	7.00	Net Photo	μmol/m <sup>2</sup> /s	5.09	2.05	6	7.99	1.89									
Fredeen et al., 1998	CA, USA	OTM	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	3 growing seasons	<i>Lasthenia californica</i>	C4	Herbaceous	Angio	A	36	Pa	94			1	7.10	4.86	9.33	1.81	6.00	Net Photo	μmol/m <sup>2</sup> /s	3.38	1.97	6	5.59	1.28									
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Dichanthium sericeum</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.51	1.14	4.45	0.62	4.00	Biomass	g	0.89	0.31	4	1.50	0.46	Water use	kgH <sub>2</sub> O/Plant	0.19	0.04	4	0.34	0.06	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Panicum coloratum</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.72	0.88	5.90	0.97	4.00	Biomass	g	4.13	0.54	4	4.09	0.31	Water use	kgH <sub>2</sub> O/Plant	0.72	0.02	4	0.71	0.07	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Leptochloa dubia</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.48	0.62	6.85	0.40	4.00	Biomass	g	1.81	0.31	4	1.66	0.39	Water use	kgH <sub>2</sub> O/Plant	0.34	0.04	4	0.25	0.06	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Pennisetum clandestinum</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.58	0.09	5.72	0.18	4.00	Biomass	g	5.48	0.31	4	6.25	0.46	Water use	kgH <sub>2</sub> O/Plant	1.20	0.04	4	1.09	0.11	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Pennisetum abpecuroides</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.38	0.26	5.55	0.48	4.00	Biomass	g	5.17	0.62	4	5.13	1.39	Water use	kgH <sub>2</sub> O/Plant	1.18	0.10	4	0.93	0.31	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Dichanthium aristatum</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.93	0.13	6.25	0.44	4.00	Biomass	g	4.67	1.70	4	6.98	1.00	Water use	kgH <sub>2</sub> O/Plant	0.94	0.33	4	1.12	0.15	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Astrebis squarrosa</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	3.59	0.13	4.58	0.66	4.00	Biomass	g	2.16	0.23	4	1.58	0.46	Water use	kgH <sub>2</sub> O/Plant	0.61	0.03	4	0.37	0.14	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Panicum decompositum</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.17	0.40	6.74	0.40	4.00	Biomass	g	1.74	0.23	4	2.86	0.46	Water use	kgH <sub>2</sub> O/Plant	0.33	0.04	4	0.43	0.09	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Astrebis pectinata</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.29	0.44	5.61	0.97	4.00	Biomass	g	2.55	0.93	4	2.20	0.46	Water use	kgH <sub>2</sub> O/Plant	0.59	0.19	4	0.39	0.06	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Eragrostis superba</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.22	1.48	6.87	1.23	4.00	Biomass	g	2.05	0.62	4	2.55	1.23	Water use	kgH <sub>2</sub> O/Plant	0.40	0.08	4	0.38	0.20	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Cenchrus ciliaris</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.83	0.13	7.88	0.75	4.00	Biomass	g	8.64	0.19	4	11.8	1.77	Water use	kgH <sub>2</sub> O/Plant	1.49	0.04	4	1.49	0.10	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Cynodon dactylon</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.62	0.22	6.39	0.48	4.00	Biomass	g	4.94	1.08	4	4.71	0.85	Water use	kgH <sub>2</sub> O/Plant	1.07	0.18	4	0.73	0.08	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Eleusine coracana</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	6.78	0.22	9.60	0.40	4.00	Biomass	g	4.86	0.23	4	8.18	1.85	Water use	kgH <sub>2</sub> O/Plant	0.72	0.04	4	0.85	0.19	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Bothriochloa bladhii</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.54	1.10	6.58	1.06	4.00	Biomass	g	1.62	0.31	4	2.47	0.46	Water use	kgH <sub>2</sub> O/Plant	0.38	0.15	4	0.41	0.11	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Bothriochloa biloba</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.68	0.57	8.52	0.75	4.00	Biomass	g	1.62	0.31	4	1.85	0.46	Water use	kgH <sub>2</sub> O/Plant	0.28	0.04	4	0.22	0.07	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Digitaria brownii</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	4.69	0.04	7.42	2.29	4.00	Biomass	g	1.62	0.89	4	2.32	0.54	Water use	kgH <sub>2</sub> O/Plant	0.35	0.03	4	0.32	0.05	4	
Ghannoum et al., 2001	Australia	GH	WUE	g DM/kg H <sub>2</sub> O	4 weeks	<i>Astrebis lappacea</i>	C4	Herbaceous	Angio	42	68	Pa	61			1	5.50	0.70	9.29	2.20	4.00	Biomass	g	2.32	0.93	4	3.74	1.00	Water use	kgH <sub>2</sub> O/Plant	0.42	0.13	4	0.40	0.03	4	
Greenep et al., 2003	New Zealand	OTC	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	14 months	<i>Pinus radiata</i>	C3	Woody	Gymno	36	66	Pa	83			1	3.39	0.31	4.81	0.13	2.00																
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O ET	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	600	μL/L	114	Nutrient		1	1.90	0.22	2.70	0.22	5.00	Biomass	g/m <sup>2</sup>	299.6	323.0	5	402.8	340.3	Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup> /0	155.0	12.24	5	149.0	4.47	5	
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O ET	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	600	μL/L	114	Nutrient		1	2.40	0.09	3.50	0.22	5.00	Biomass	g/m <sup>2</sup>	389.8	628.8	5	552.2	298.1	Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup> /0	160.0	12.24	5	157.0	4.47	5	
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O ET	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	600	μL/L	114	Nutrient		1	3.50	0.22	5.70	0.22	5.00	Biomass	g/m <sup>2</sup>	583.2	400.3	5	941.9	69.2	Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup> /0	165.0	12.24	5	165.0	4.47	5	

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XNE	XSA	XNE	Parameter Y	Units Y	YA	YSA	YNA	YNE	YSA	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZSA	ZNE				
2003																							3	9	4	4				0											
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O E	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	400	μL/L	43	Nutrient		1.90	0.225	2.70	0.225	0.00	Biomass	g/m <sup>2</sup>	299.3	23.05	397.4	46.15			Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup>	155.0	0.2	24.5	0	148.0	0.2	24.5	0			
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O E	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	400	μL/L	43	Nutrient		2.40	0.095	3.60	0.225	0.00	Biomass	g/m <sup>2</sup>	389.2	8.85	572.9	5.05			Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup>	160.0	0.2	24.5	0	159.0	0.4	47.5	0			
Grunzweig et al., 2003	Switzerland	GC	WUE	g total dry wt. kg <sup>-1</sup> H <sub>2</sub> O E	100 days	<i>mix culture</i>	C3	Herbaceous	Angio	280	400	μL/L	43	Nutrient		3.50	0.225	4.90	0.225	0.00	Biomass	g/m <sup>2</sup>	583.2	40.35	805.1	5.05			Evapotrans	kgH <sub>2</sub> O/m <sup>2</sup>	165.0	0.2	24.5	0	163.0	0.4	47.5	0			
Jensen and Christensen, 2004	Denmark	GC	WUE	g shoot dry matter (DM)/kg water used	80 days	<i>Triticum aestivum</i>	C4	Crop	Angio	365	500	ppm	37	Nutrient		6.50	0.124	8.64	0.384	0.00																					
Jensen and Christensen, 2004	Denmark	GC	WUE	g shoot dry matter (DM)/kg water used	80 days	<i>Triticum aestivum</i>	C4	Crop	Angio	365	500	ppm	37	Nutrient		7.70	0.444	9.92	0.684	0.00																					
Jensen and Christensen, 2004	Denmark	GC	WUE	g shoot dry matter (DM)/kg water used	80 days	<i>Triticum aestivum</i>	C4	Crop	Angio	365	500	ppm	37	Nutrient		8.24	0.244	10.9	0.624	0.00																					
Lodge et al., 2001	FA, USA	OTC	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	3 growing seasons	<i>Quercus myrtifolia</i>	C3	Woody	Angio	377	724	μmol/mol	92			1.98	0.537	5.99	1.807	0.00	Net Photo	μmol/m <sup>2</sup> /s	9.80	14.76	15.70	4.656															
Manderscheid and Weigel, 2007	Germany	OTC	WUE	g/Kg	2 growing seasons	<i>Triticum aestivum</i>	C4	Crop	Angio	A	A+280	ppm	74	H <sub>2</sub> O		4.08	0.464	4.60	0.584	0.00																					
Norby et al., 1986	TN, USA	GC	WUE	g/L	40 weeks	<i>Quercus alba</i>	C3	Woody	Angio	362	690	μL/L	91			3.83	1.646	6.10	1.966	0.00																					
Pettersen and McDonald, 1992	Sweden	GC	ITE	μmol/mol	70 days	<i>Betula pendula</i>	C3	Woody	Angio	350	700	μmol/mol	100			2.17	1.117	3.54	0.287	0.00	Net Photo	μmol/m <sup>2</sup> /s	12.48	0.875	15.94	1.195															
Robredo et al., 2007	Spain	GC	ITE	μmolCO <sub>2</sub> /mmol H <sub>2</sub> O	1 growing season	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O		4.10	0.313	12.10	1.233	0.00																					
Robredo et al., 2007	Spain	GC	WUE	g DW/kg H <sub>2</sub> O transpired	1 growing season	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O		3.70	0.053	9.60	0.873	0.00																					
Roumet et al., 2000	France	GH	WUE	mmol/mol	6 months	<i>Bromus madritensis</i>	C4	Herbaceous	Angio	350	700	μmol/mol	100			0.120	0.034	0.21	0.034	0.00	Net Photo	μmol/m <sup>2</sup> /s	10.53	2.874	12.03	1.694			St cond	mmol/m <sup>2</sup> /s	92.5	34.684	60.6	423.404							
Roumet et al., 2000	France	GH	WUE	mmol/mol	6 months	<i>Bromus erectus</i>	C4	Herbaceous	Angio	350	700	μmol/mol	100			0.090	0.014	0.17	0.034	0.00	Net Photo	μmol/m <sup>2</sup> /s	8.32	1.354	6.62	2.024			St cond	mmol/m <sup>2</sup> /s	96.8	31.924	47.8	14.894							
Roumet et al., 2000	France	GH	WUE	mmol/mol	6 months	<i>Medicago minima</i>	C3	Herbaceous	Angio	350	700	μmol/mol	100			0.080	0.014	0.09	0.014	0.00	Net Photo	μmol/m <sup>2</sup> /s	8.38	3.204	8.45	2.364			St cond	mmol/m <sup>2</sup> /s	164.8	72.344	93.6	21.284							
Roumet et al., 2000	France	GH	WUE	mmol/mol	6 months	<i>Medicago glomerata</i>	C3	Herbaceous	Angio	350	700	μmol/mol	100			0.080	0.014	0.09	0.034	0.00	Net Photo	μmol/m <sup>2</sup> /s	7.01	2.194	8.18	2.364			St cond	mmol/m <sup>2</sup> /s	139.3	34.684	91.4	51.084							
Tezara et al., 2002	Venezuela	GC	WUE	mmol/mol	52 days	<i>Helianthus annuus</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O		3.18	0.516	8.67	0.426	0.00	Net Photo	μmol/m <sup>2</sup> /s	19.82	4.96	28.99	1.936															
Vu, 2005	FL, USA	GH	ITE	mmol/mol	3 months	<i>Arachis hypogaea</i>	C3	Herbaceous	Angio	360	720	μmol/mol	100	Temperature		4.50	0.8217	7.00	1.6517	0.00	Net Photo	μmol/m <sup>2</sup> /s	32.404	12.17	43.80	7.0117			Transpiration	mmol/m <sup>2</sup> /s	7.20	1.24	17.6	30	1.65	17					
Vu, 2005	FL, USA	GH	ITE	mmol/mol	3 months	<i>Arachis hypogaea</i>	C3	Herbaceous	Angio	360	720	μmol/mol	100	Temperature		4.40	1.2417	6.20	1.2417	0.00	Net Photo	μmol/m <sup>2</sup> /s	34.204	54.17	39.90	7.4217			Transpiration	mmol/m <sup>2</sup> /s	7.80	1.65	17.6	50	1.65	17					
Watling and Press, 2000	UK	GC	WUE	μmolCO <sub>2</sub> /mmolH <sub>2</sub> O	80 days	<i>Oryza sativa</i>	C4	Crop	Angio	350	700	μmol/mol	100			3.33	0.683	5.09	0.293	0.00																					
Watling and Press, 1997	UK	GC	WUE	mmol/mol	60 days	<i>Sorghum bicolor</i>	C4	Crop	Angio	350	700	μmol/mol	100			0.180	0.0410	0.55	0.0810	0.00	Net Photo	μmol/m <sup>2</sup> /s	16.64	0.7710	26.32	1.9210			St cond	mol/m <sup>2</sup> /s	0.09	0.00	10.0	0.05	0.01	10					
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Caragana korshinskii</i>	C3	Woody	Angio	400	800	μmol/mol	100	Temperature		8.20	6.004	14.60	6.004	0.00									Transpiration	g/d	4.50	1.40	4.0	2.80	2.40	4.0					
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Caragana korshinskii</i>	C3	Woody	Angio	400	800	μmol/mol	100			5.80	1.404	10.70	5.004	0.00									Transpiration	g/d	5.70	2.40	4.0	4.00	1.40	4.0					
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Hedysarum laeve</i>	C3	Herbaceous	Angio	400	800	μmol/mol	100	Temperature		3.10	0.804	6.40	3.804	0.00										Transpiration	g/d	18.60	1.00	4.0	8.40	2.40	4.0				
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Hedysarum laeve</i>	C3	Herbaceous	Angio	400	800	μmol/mol	100			2.70	1.804	4.20	1.204	0.00										Transpiration	g/d	18.30	4.20	4.0	14.30	6.0	4.0				
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Artemisia sphaerocephala</i>	C4	Woody	Angio	400	800	μmol/mol	100	Temperature		3.30	1.804	5.50	0.604	0.00											Transpiration	g/d	24.50	7.00	4.0	23.90	4.20	4.0			
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Artemisia sphaerocephala</i>	C4	Woody	Angio	400	800	μmol/mol	100			3.40	1.404	6.30	3.204	0.00											Transpiration	g/d	24.30	6.60	4.0	15.60	8.40	4.0			
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Artemisia ordosica</i>	C4	Woody	Angio	400	800	μmol/mol	100	Temperature		3.10	1.204	4.40	0.404	0.00												Transpiration	g/d	26.70	6.40	4.0	22.60	7.40	4.0		
Zheng et al., 2010	Japan	GC	WUE	g/kg	8 weeks	<i>Artemisia ordosica</i>	C4	Woody	Angio	400	800	μmol/mol	100			3.00	1.204	4.10	1.804	0.00												Transpiration	g/d	25.20	9.40	4.0	23.00	7.80	4.0		
Aranjuelo et al., 2006	Spain	GT	WUE	mg/gH <sub>2</sub> O	60 days x2	<i>Medicago sativa</i>	C3	Crop	Angio	400	720	μmol/mol	80	H <sub>2</sub> O		4.00	0.718	4.00	1.138	0.00	Biomass	g DW/p	2.82	0.278	2.69	0.828			Water use	ml H <sub>2</sub> O/p	700.5	69.168	684.9	37.988							

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XE	XSE	XNE	Parameter Y	Units Y	YA	YSA	YNA	YE	YSE	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZSE	ZNE
Aranjuelo et al., 2006	Spain	GI	WUE	mg/gH <sub>2</sub> O	60 days x2	<i>Medicago sativa</i>	C3	Crop	Angio	400	720	μmol/mol	80	H <sub>2</sub> O			3.80	1.70	6.63	3.17	8.00	Biomass	g DW/p	2.72	1.27	8	3.85	1.45	8	Water use	ml H <sub>2</sub> O/p	111.14	7.98	585.85	9.18	3	
Aranjuelo et al., 2006	Spain	GI	ITE	μmol CO <sub>2</sub> /mmol H <sub>2</sub> O	46 days x2	<i>Medicago sativa</i>	C3	Crop	Angio	400	720	μmol/mol	80	H <sub>2</sub> O			0.07	0.03	12	0.13	0.08	12.00	Net Photo	μmol/m <sup>2</sup> /s	13.80	3.48	12	15.90	6.24	12							
Aranjuelo et al., 2006	Spain	GI	ITE	μmol CO <sub>2</sub> /mmol H <sub>2</sub> O	46 days x2	<i>Medicago sativa</i>	C3	Crop	Angio	400	720	μmol/mol	80	H <sub>2</sub> O			0.13	0.03	12	0.21	0.09	12.00	Net Photo	μmol/m <sup>2</sup> /s	15.50	4.85	12	20.90	5.20	12							
Wu et al., 2004	China	GC	WUE	g/kg	93 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μL/L	100	H <sub>2</sub> O			1.09	0.22	1.76	0.78	6.00	Biomass	g/plant	0.85	0.05	6	1.61	0.60	6								
Liret al., 2003	China	GC	WUE	kg/m <sup>2</sup>	40 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O	Nitrogen		1.92	0.48	1.99	0.13	3.00								EvapoTrans	kg/pot	1.94	0.04	3	1.59	0.09	3	
Liret al., 2003	China	GC	WUE	kg/m <sup>2</sup>	40 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O	Nitrogen		2.93	0.07	3.23	0.10	3.00								EvapoTrans	kg/pot	4.51	0.11	3	4.02	0.13	3	
Liret al., 2003	China	GC	WUE	kg/m <sup>2</sup>	40 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O	Nitrogen		3.08	0.10	3.40	0.20	3.00								EvapoTrans	kg/pot	5.86	0.20	3	5.61	0.24	3	
Liret al., 2003	China	GC	WUE	kg/m <sup>2</sup>	40 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O	Nitrogen		3.23	0.18	3.86	0.07	3.00								EvapoTrans	kg/pot	6.10	0.09	3	5.93	0.15	3	
Liret al., 2003	China	GC	WUE	kg/m <sup>2</sup>	40 days	<i>Triticum aestivum</i>	C4	Crop	Angio	350	700	μmol/mol	100	H <sub>2</sub> O	Nitrogen		3.23	0.28	3.87	0.07	3.00								EvapoTrans	kg/pot	5.32	0.11	3	5.48	0.07	3	
Franzaring et al., 2011	Germany	GC	WUE	g DMI	106 days	<i>Brassica napus</i>	C4	Crop	Angio	380	550	ppm	45	Nitrogen			3.02	0.12	3.66	0.19	6.00																
Franzaring et al., 2011	Germany	GC	WUE	g DMI	106 days	<i>Brassica napus</i>	C4	Crop	Angio	380	550	ppm	45	Nitrogen			3.50	0.21	4.80	0.38	6.00																
Franzaring et al., 2011	Germany	GC	WUE	g DMI	106 days	<i>Brassica napus</i>	C4	Crop	Angio	380	550	ppm	45	Nitrogen			3.89	0.20	4.97	0.30	6.00																
Vu et al., 2006	FL, USA	GH	ITE	mmol CO <sub>2</sub> /mol H <sub>2</sub> O	2 years	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	μmol/mol	100				3.64	1.93	5.23	0.71	5.00	Photosynthesis	μmol/m <sup>2</sup> /s	14.18	0.35	15.08	2.02	5									
Gomissen et al., 1995	Netherlands	GH	WUE	g/L	14 months	<i>Pseudotsuga menziesii</i>	C3	Woody	Gymno	350	700	μmol/mol	100				5.50	0.89	7.20	0.89	6.00								Water use	ml	2340.00	243.46	1870.00	243.46	3		
Kim et al., 2006	MD, USA	GC	ITE	μmol CO <sub>2</sub> /mmol H <sub>2</sub> O	71 days	<i>Zea mays</i>	C4	Crop	Angio	370	750	μmol/mol	103				6.09	0.93	12.58	0.99	3.00	Photosynthesis	μmol/m <sup>2</sup> /s	48.72	2.54	50.63	2.54	3	Transpiration	mmol/m <sup>2</sup> /s	8.61	0.96	3	4.05	0.96	3	
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Agrostemma githago</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				70.02	5.05	157.32	5.05	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Centaurea cyanus</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				55.02	5.05	127.32	5.05	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Chenopodium album</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				57.95	5.05	90.00	27.95	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Euphorbia helioscopia</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				50.05	5.00	77.50	10.03	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Papaver rhoeas</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				67.92	5.04	120.00	20.04	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Persicaria maculosa</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				50.05	5.00	67.50	0.00	1.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Sinapis arvensis</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				65.02	5.04	147.91	17.94	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Thlaspi arvense</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				65.02	5.05	102.91	17.95	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Tripleurospermum perforatum</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				55.05	5.00	112.33	7.94	0.00																
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	<i>Triticum aestivum</i>	C4	Herbaceous	Angio	A	A+150	μmol/mol	39				77.95	5.00	142.37	5.04	0.00																
Ferns and Taylor, 1995	UK	GH	WUE	g DW/kg H <sub>2</sub> O	37 days	<i>Sanguisorba minor Scop</i>	C3	Herbaceous	Angio	A	590	μmol/mol	65	H <sub>2</sub> O			3.25	0.94	4.01	0.82	4.00								Water loss	g H <sub>2</sub> O/g SDW	464.30	230.04	325.97	0.004	7		
Ferns and Taylor, 1995	UK	GH	WUE	g DW/kg H <sub>2</sub> O	37 days	<i>Anthyllis vulneraria</i>	C3	Herbaceous	Angio	A	590	μmol/mol	65	H <sub>2</sub> O			1.94	0.44	2.81	0.42	4.00								Water loss	g H <sub>2</sub> O/g SDW	654.30	315.44	469.07	0.404	7		
Ferns and Taylor, 1995	UK	GH	ITE	mmol CO <sub>2</sub> /mol H <sub>2</sub> O	37 days	<i>Sanguisorba minor Scop</i>	C3	Herbaceous	Angio	A	590	μmol/mol	65	H <sub>2</sub> O			2.92	0.59	4.26	0.29	5.00	Photosynthesis	μmol/m <sup>2</sup> /s	4.20	0.17	5.05	0.41	5	Transpiration	mmol/m <sup>2</sup> /s	1.46	0.22	5	1.20	0.07	5	
Ferns and Taylor, 1995	UK	GH	ITE	mmol CO <sub>2</sub> /mol H <sub>2</sub> O	37 days	<i>Anthyllis vulneraria</i>	C3	Herbaceous	Angio	A	590	μmol/mol	65	H <sub>2</sub> O			3.38	0.78	4.13	0.69	5.00	Photosynthesis	μmol/m <sup>2</sup> /s	5.48	0.73	6.29	0.29	5	Transpiration	mmol/m <sup>2</sup> /s	1.65	0.21	5	1.53	0.10	5	
Khurana and Singh, 2004	India	Trench	ITE	μmol/mol	30 days	<i>Albizia procera</i>	C3	Woody	Angio	A	700	ppm	84	Seed size			396.60	60.03	846.39	101.30	0.00	Photosynthesis	μmol/m <sup>2</sup> /s	4.90	0.07	9.50	0.05	3	Transpiration	mmol/m <sup>2</sup> /s	12.30	0.35	3	11.18	0.69	3	
Khurana and Singh, 2004	India	Trench	ITE	μmol/mol	30 days	<i>Acacia nilotica</i>	C3	Woody	Angio	A	700	ppm	84	Seed size			344.46	46.43	739.66	60.03	0.00	Photosynthesis	μmol/m <sup>2</sup> /s	4.66	0.09	9.83	0.05	3	Transpiration	mmol/m <sup>2</sup> /s	13.50	0.02	3	13.28	0.02	3	

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XE	XSE	XNE	Parameter Y	Units Y	YA	YSA	YNA	YE	YSE	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZSE	ZNE	
2004																81	5	0	1				/s								s							
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Phyllanthus emblica</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		449	126	3	656	42	13	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.85	0.09	3	8.19	0.02	3	Transpiration	mmol/m <sup>2</sup> /s	13.32	1.56	3	12.50	0.61	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Terminalia arjuna</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		333	60	43	978	325	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.70	0.7	13	11.09	0.99	3	Transpiration	mmol/m <sup>2</sup> /s	11.16	0.35	3	10.34	0.50	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Terminalia chebula</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		236	20	23	448	102	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.76	0.09	3	5.39	0.03	3	Transpiration	mmol/m <sup>2</sup> /s	11.60	0.52	3	10.00	0.87	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Albizia procera</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		342	84	83	1017	71	33	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.88	0.10	3	9.64	0.06	3	Transpiration	mmol/m <sup>2</sup> /s	12.00	0.47	3	9.49	0.87	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Acacia nilotica</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		305	45	83	874	424	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	4.08	0.09	3	10.27	0.03	3	Transpiration	mmol/m <sup>2</sup> /s	13.34	0.10	3	11.90	1.09	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Phyllanthus emblica</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		367	72	33	943	106	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.02	0.10	3	10.16	0.05	3	Transpiration	mmol/m <sup>2</sup> /s	13.80	1.04	3	10.74	0.52	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Terminalia arjuna</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		216	62	73	627	303	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.54	0.90	3	7.34	0.83	3	Transpiration	mmol/m <sup>2</sup> /s	11.82	0.43	3	11.52	0.81	3
Khurana and Singh, 2004	India	Trench	ITE	umol/mol	30 days	<i>Terminalia chebula</i>	C3	Woody	Angio	A	700	ppm	84	Seed size		241	12	43	654	352	3	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.58	0.03	3	7.73	0.19	3	Transpiration	mmol/m <sup>2</sup> /s	10.70	0.85	3	10.20	1.00	3
Murthy et al., 2005	AZ, USA	Mesocosm	ITE	umol/mol	14 days	<i>Populus deltoides</i>	C3	Woody	Angio	43	120	Pa	179	H <sub>2</sub> O	VPD	3.89	0.186		4.62	0.496	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.59	1.116		25.00	2.236		Transpiration	mmol/m <sup>2</sup> /s	5.39	0.44	6	3.69	0.33	6	
Murthy et al., 2005	AZ, USA	Mesocosm	ITE	umol/mol	14 days	<i>Populus deltoides</i>	C3	Woody	Angio	43	120	Pa	179	H <sub>2</sub> O	VPD	2.64	0.386		3.74	0.386	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.59	1.676		20.00	1.676		Transpiration	mmol/m <sup>2</sup> /s	5.39	0.66	6	3.78	0.44	6	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		3.90	0.343		5.22	0.593	0.00									Transpiration	g H <sub>2</sub> O/p	244.41	11.603		211.41	1.093		
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		4.59	0.513		6.69	0.843	0.00									Transpiration	g H <sub>2</sub> O/p	166.91	10.93		152.8	8.14	3	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		5.28	0.683		8.16	1.183	0.00									Transpiration	g H <sub>2</sub> O/p	122.09	7.70	3	105.47	1.10	3	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		5.63	0.783		9.05	1.183	0.00									Transpiration	g H <sub>2</sub> O/p	93.40	6.41	3	83.24	4.85	3	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		3.54	0.423		4.71	0.513	0.00									Transpiration	g H <sub>2</sub> O/p	250.61	6.63		222.8	1.1283		
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		4.62	0.593		5.79	0.593	0.00									Transpiration	g H <sub>2</sub> O/p	169.21	10.53		167.7	9.97	3	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		4.77	0.593		6.73	0.783	0.00									Transpiration	g H <sub>2</sub> O/p	130.08	8.14	3	115.85	8.9	3	
Perez-Lopez et al., 2009	Spain	GC	WUE	g DW/kg H <sub>2</sub> O	28 days	<i>Hordeum vulgare</i>	C4	Crop	Angio	350	700	umol/mol	100	NaCl		5.32	0.783		7.51	1.103	0.00									Transpiration	g H <sub>2</sub> O/p	95.10	6.93	3	89.20	5.72	3	
Retuerto and Woodward, 1993	UK	GC	WUE	mg/gH <sub>2</sub> O	2 months	<i>Sinapis alba</i>	C4	Crop	Angio	350	700	ppm	100	H <sub>2</sub> O	Wind	1.04	0.573		1.71	0.693	0.00	Biomass	mg	757.3	362	3	1216	471	3									
Retuerto and Woodward, 1993	UK	GC	WUE	mg/gH <sub>2</sub> O	2 months	<i>Sinapis alba</i>	C4	Crop	Angio	350	700	ppm	100	H <sub>2</sub> O	Wind	0.37	0.183		0.50	0.173	0.00	Biomass	mg	374.7	417	23	522.2	201	3									
Seneweera et al., 1998	Australia	GC	WUE	gDW/L H <sub>2</sub> O	39	<i>Panicum coloratum</i>	C4	Herbaceous	Angio	350	1000	uL/L	186	H <sub>2</sub> O	VPD	1.73	0.144		2.68	0.334	0.00	Biomass	g/plant	2.07	0.174		3.21	0.214										
Seneweera et al., 1998	Australia	GC	WUE	gDW/L H <sub>2</sub> O	39	<i>Panicum coloratum</i>	C4	Herbaceous	Angio	350	1000	uL/L	186	H <sub>2</sub> O	VPD	2.48	0.194		2.43	0.124	0.00	Biomass	g/plant	3.15	0.194		3.08	0.174										
Tuba et al., 1996	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	11 months	<i>Festuca rupicola</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			0.67	0.135		2.58	0.565	0.00																	
Tuba et al., 1996	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	11 months	<i>Dactylis glomerata</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			1.29	0.195		1.78	0.375	0.00																	
Tuba et al., 1996	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	11 months	<i>Filipendula vulgaris</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			1.13	0.175		4.71	0.875	0.00																	
Tuba et al., 1996	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	11 months	<i>Salvia nemorosa</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			2.43	0.615		4.77	0.635	0.00																	
Vu and Allen, 2009	FL, USA	GH	ITE	mmol CO <sub>2</sub> /mol H <sub>2</sub> O	5 months	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	umol/mol	100	H <sub>2</sub> O		6.06	0.584		8.11	0.784	0.00	Photosynthesis	umol/m <sup>2</sup> /s	31.32	4.34		31.68	3.124										
DeLuis et al., 1999	Spain	GC	WUE	mg/gH <sub>2</sub> O	30 days	<i>Medicago sativa</i>	C3	Crop	Angio	400	700	umol/mol	75	H <sub>2</sub> O		1.54	0.4818		4.61	0.6418	0	Biomass	g	0.22	0.0218		0.80	0.0418		Water consumption	g	143290	1.118		1692180	2.18		
Picon et al., 1996	France	GH	WUE	mg/gH <sub>2</sub> O	320 days	<i>Quercus robur</i>	C3	Woody	Angio	350	700	umol/mol	100	H <sub>2</sub> O		7.41	1.248		10.99	3.028	0.00																	
Sherwin et al., 2013	Australia	GH	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	400	uL/L	43	Temperature		0.78	0.325		1.95	0.475	0.00									Transpiration	mmol/m <sup>2</sup> /s	6.79	0.53	5	5.76	0.89	5	

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XE	XSA	XNE	Parameter Y	Units Y	YA	YSA	YNA	YE	YSA	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZSA	ZNE
Sherwin et al., 2013	Australia	GH	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	400	uL/L	43	Temperature		0.78	0.325	4.40	0.375	0.00			Transpiration	mmol/m <sup>2</sup> /s								6.79	0.53	5	5.52	0.71	5
Sherwin et al., 2013	Australia	GH	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	640	uL/L	129	Temperature		0.54	0.745	1.18	0.325	0.00			Transpiration	mmol/m <sup>2</sup> /s								4.65	0.80	5	8.38	1.06	5
Sherwin et al., 2013	Australia	GH	ITE	mmolCO <sub>2</sub> /molH <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	640	uL/L	129	Temperature		0.54	0.745	2.26	0.475	0.00			Transpiration	mmol/m <sup>2</sup> /s								4.65	0.80	5	7.94	0.97	5
Sherwin et al., 2013	Australia	GH	WUE	gDM/kg H <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	400	uL/L	43	Temperature		2.38	0.205	2.95	0.325	0.00	Biomass	g/plant	1.41	0.795	2.41	1.325	Water use	kg/plant	0.49	0.15	5	0.52	0.21	5			
Sherwin et al., 2013	Australia	GH	WUE	gDM/kg H <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	400	uL/L	43	Temperature		2.38	0.205	3.96	0.325	0.00	Biomass	g/plant	1.41	0.795	6.94	1.325	Water use	kg/plant	0.49	0.15	5	0.38	0.05	5			
Sherwin et al., 2013	Australia	GH	WUE	gDM/kg H <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	640	uL/L	129	Temperature		2.18	0.365	2.16	0.205	0.00	Biomass	g/plant	1.29	0.795	3.35	1.055	Water use	kg/plant	0.67	0.36	5	0.66	0.12	5			
Sherwin et al., 2013	Australia	GH	WUE	gDM/kg H <sub>2</sub> O	59 days	<i>Eucalyptus saligna</i>	C3	Woody	Angio	280	640	uL/L	129	Temperature		2.18	0.365	2.66	0.405	0.00	Biomass	g/plant	1.29	0.795	10.06	1.845	Water use	kg/plant	0.67	0.36	5	0.55	0.10	5			
Chen et a., 1999	Germny	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	2 months	<i>Solanum muricatum</i>	C3	Woody	Angio	350	700	ppm	100	NaCl		3.31	0.0415	6.12	0.0415	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.30	0.2015	14.50	0.4015	Transpiration	mmolH <sub>2</sub> O/m <sup>2</sup> /s	2.51	0.0215	2.36	0.0215					
Chen et a., 1999	Germny	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	2 months	<i>Solanum muricatum</i>	C3	Woody	Angio	350	700	ppm	100	NaCl		2.34	0.0415	4.98	0.0915	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.40	0.1015	10.70	0.2015	Transpiration	mmolH <sub>2</sub> O/m <sup>2</sup> /s	2.29	0.0315	2.14	0.0215					
Chen et a., 1999	Germny	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	2 months	<i>Solanum muricatum</i>	C3	Woody	Angio	350	1050	ppm	200	NaCl		3.31	0.0415	7.41	0.1015	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.30	0.2015	14.30	0.3015	Transpiration	mmolH <sub>2</sub> O/m <sup>2</sup> /s	2.51	0.0215	1.93	0.0515					
Chen et a., 1999	Germny	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	2 months	<i>Solanum muricatum</i>	C3	Woody	Angio	350	1050	ppm	200	NaCl		2.34	0.0415	5.78	0.0415	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.40	0.1015	10.30	0.2015	Transpiration	mmolH <sub>2</sub> O/m <sup>2</sup> /s	2.29	0.0315	1.79	0.0415					
Clifford et al., 2000	UK	GH	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	4 months	<i>Arachis hypogaea</i>	C3	Herbaceous	Angio	375	700	umol/mol	87	H <sub>2</sub> O	Temperature	3.00	1.125	5.50	2.465	0.00	Photosynthesis	umol/m <sup>2</sup> /s	19.90	1.795	35.20	4.025	Transpiration	mmolH <sub>2</sub> O/m <sup>2</sup> /s	7.40	2.24	5	6.50	1.57	5			
Zhang et al., 2008	China	CTC	ITE	mmol/mol	6 months	<i>Betula albosinensis</i>	C3	Woody	Angio	350	700	umol/mol	100	Planting density		3.30	0.523	6.10	0.693	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.80	1.563	6.90	0.873	Transpiration	mmol/m <sup>2</sup> /s	2.70	0.35	3	1.10	0.17	3			
Zhang et al., 2008	China	CTC	ITE	mmol/mol	7 months	<i>Betula albosinensis</i>	C3	Woody	Angio	350	700	umol/mol	100	Planting density		4.10	0.873	5.90	1.213	0.00	Photosynthesis	umol/m <sup>2</sup> /s	7.30	0.393	6.50	0.523	Transpiration	mmol/m <sup>2</sup> /s	1.80	0.52	3	1.10	0.52	3			
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Acer saccharum</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	0.88	0.396	1.79	0.766	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.59	1.306	5.37	2.726											
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Platanus occidentalis</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	1.21	0.126	2.51	0.206	0.00	Photosynthesis	umol/m <sup>2</sup> /s	14.4	1.426	19.38	7.26											
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	0.96	0.226	2.16	0.346	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.5	2.136	24.46	0.06											
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Acer saccharum</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	1.60	0.176	2.93	0.696	0.00	Photosynthesis	umol/m <sup>2</sup> /s	4.09	1.676	6.22	2.746											
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Platanus occidentalis</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	1.09	0.096	2.91	0.866	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.0	3.146	18.63	3.776											
Tschaplinski et al., 1995	TN, USA	OTC	ITE	mmol/mol	3 months	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+300	umol/mol	79	H <sub>2</sub> O	H <sub>2</sub> O	1.36	0.346	3.27	1.086	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.9	4.316	23.87	6.306											
Qaderi and Reid, 2005	Canada	GC	ITE	umol/mmol	1 month X 4	<i>Brassica napus</i>	C4	Crop	Angio	370	740	umol/mol	100	UV		2.39	0.176	3.92	0.176	0.00	Photosynthesis	umol/m <sup>2</sup> /s	7.71	1.296	11.46	1.406	Transpiration	mmol/m <sup>2</sup> /s	3.30	0.78	6	2.94	0.44	6			
Qaderi and Reid, 2005	Canada	GC	ITE	umol/mmol	1 month X 4	<i>Brassica napus</i>	C4	Crop	Angio	370	740	umol/mol	100	UV		2.58	0.176	4.47	0.206	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.13	1.226	12.26	1.136	Transpiration	mmol/m <sup>2</sup> /s	3.19	0.61	6	2.76	0.34	6			
Polley et al., 1996	TX, USA	GH	WUE	g/L	2 growing seasons	<i>Atriplex canescens</i>	C4	Woody	Angio	360	680	umol/mol	89			4.42	10.144	5.07	0.444	0.00	Biomass	g	141.422	84.0	95.30	11.040											
Polley et al., 1996	TX, USA	GH	WUE	g/L	2 growing seasons	<i>Schizachyrium scoparium</i>	C4	Herbaceous	Angio	360	680	umol/mol	89			10.34	8.065	12.92	1.596	0.00	Biomass	g	81.902	3.063	131.636	2.85											
Polley et al., 1996	TX, USA	GH	WUE	g/L	2 growing seasons	<i>Atriplex canescens</i>	C4	Woody	Angio	360	1005	umol/mol	179			4.42	10.144	4.93	0.444	0.00	Biomass	g	141.422	84.0	137.91	11.040											
Polley et al., 1996	TX, USA	GH	WUE	g/L	2 growing seasons	<i>Schizachyrium scoparium</i>	C4	Herbaceous	Angio	360	1005	umol/mol	179			10.34	8.065	11.83	1.596	0.00	Biomass	g	81.902	3.063	143.736	2.85											
Centritto et al., 2002	UK	OTC	ITE	mmol/mol	370 days	<i>Prunus persica</i>	C3	Woody	Angio	350	700	umol/mol	100	H <sub>2</sub> O		3.39	0.773	5.36	0.483	0.00	Photosynthesis	umol/m <sup>2</sup> /s	11.03	0.893	16.70	1.073											
Centritto et al., 2002	UK	OTC	ITE	mmol/mol	370 days	<i>Prunus persica</i>	C3	Woody	Angio	350	700	umol/mol	100	H <sub>2</sub> O		5.16	2.265	7.81	0.825	0.00									Water uptake	kg/plant	12.32	1.59	5	12.7	1.59	5	
Magliulo et al., 2003	Italy	FACE	WUE	g/L	2 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	367	552	ppm	50			3.09	0.346	5.14	1.406	0.00	Biomass belowground	g/m <sup>2</sup>	1018	53.8600	1446	213.511	Water use	L/m <sup>2</sup>	330.0	119.606	281.030	130.626					
Zhang and Dang, 2006	Canada	GH	ITE	umol/mmol	3.5 months	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	ppm	100	Nutrient		3.94	0.896	9.15	2.976	0.00	Photosynthesis	umol/m <sup>2</sup> /s	14.2	0.896	20.83	2.976	Transpiration	mmol/m <sup>2</sup> /s	3.62	0.89	6	2.30	2.97	6			
Zhang and Dang, 2006	Canada	GH	ITE	umol/mmol	3.5 months	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	ppm	100	Nutrient		3.09	1.486	4.85	3.276	0.00	Photosynthesis	umol/m <sup>2</sup> /s	7.59	1.486	10.48	3.276	Transpiration	mmol/m <sup>2</sup> /s	2.58	1.48	6	2.30	3.27	6			
Zhang and Dang, 2006	Canada	GH	ITE	umol/mmol	4 months	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	ppm	100	Soil Temp		4.16	1.273	4.10	1.083	0.00	Photosynthesis	umol/m <sup>2</sup> /s	7.38	2.443	10.78	1.833	Transpiration	mmol/m <sup>2</sup> /s	1.85	0.86	3	2.66	0.36	3			

References	Location	Facility	Parameter X	Units X	Duration	Species	Control C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XNE	XSE	XNE	Parameter Y	Units Y	YA	YSA	YNA	YNE	YSE	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZNE	ZSE	ZNE			
2005																						/s								s										
Zhang and Dang, 2005	Canada	GH	ITE	umol/mmol	4 months	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	ppm	100	Soil Temp			2.81	0.323	3.91	1.383	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.91	1.833	13.7	3.453		Transpiration	mmol/m <sup>2</sup> /s		3.18	0.86	3	3.55	0.50	3			
Zhang and Dang, 2005	Canada	GH	ITE	umol/mmol	4 months	<i>Betula papyrifera</i>	C3	Woody	Angio	360	720	ppm	100	Soil Temp			3.27	0.693	4.55	1.183	0.00	Photosynthesis	umol/m <sup>2</sup> /s	9.38	1.423	11.99	2.443		Transpiration	mmol/m <sup>2</sup> /s		2.90	0.90	3	2.63	0.68	3			
Zhang and Dang, 2005	Canada	GH	ITE	umol/mmol	4 months	<i>Pinus banksiana</i>	C3	Woody	Gymno	360	720	ppm	100	Soil Temp			4.03	0.893	5.93	1.323	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.83	2.843	23.09	10.93		Transpiration	mmol/m <sup>2</sup> /s		3.50	1.22	3	3.91	1.54	3			
Zhang and Dang, 2005	Canada	GH	ITE	umol/mmol	4 months	<i>Pinus banksiana</i>	C3	Woody	Gymno	360	720	ppm	100	Soil Temp			3.67	0.373	5.96	0.893	0.00	Photosynthesis	umol/m <sup>2</sup> /s	17.58	1.013	23.09	7.713		Transpiration	mmol/m <sup>2</sup> /s		4.83	0.36	3	3.94	1.54	3			
Zhang and Dang, 2005	Canada	GH	ITE	umol/mmol	4 months	<i>Pinus banksiana</i>	C3	Woody	Gymno	360	720	ppm	100	Soil Temp			3.73	0.693	6.14	3.283	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.14	4.73	20.63	11.53		Transpiration	mmol/m <sup>2</sup> /s		4.38	1.85	3	3.76	3.30	3			
Zaghdoud et al., 2013	Tunasia	GC	ITE	umol/mmol	5 weeks	<i>Brassica oleracea</i>	C3	Crop	Angio	380	800	ppm	111	NaCl			2.25	0.145	5.01	1.045	0.00	Photosynthesis	umol/m <sup>2</sup> /s	6.72	0.635	9.74	0.445		Transpiration	mmol/m <sup>2</sup> /s		2.96	0.21	5	1.99	0.47	5			
Zaghdoud et al., 2013	Tunasia	GC	ITE	umol/mmol	5 weeks	<i>Brassica oleracea</i>	C3	Crop	Angio	380	800	ppm	111	NaCl			3.08	0.385	6.57	0.615	0.00	Photosynthesis	umol/m <sup>2</sup> /s	6.78	0.635	10.79	0.955		Transpiration	mmol/m <sup>2</sup> /s		2.29	0.46	5	1.62	0.17	5			
Zaghdoud et al., 2013	Tunasia	GC	ITE	umol/mmol	5 weeks	<i>Brassica oleracea</i>	C3	Crop	Angio	380	800	ppm	111	NaCl			2.91	0.585	9.12	0.425	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.65	1.135	10.53	0.575		Transpiration	mmol/m <sup>2</sup> /s		1.96	0.32	5	1.16	0.06	5			
Ward et al., 1996	South Africa	OTC	ITE	umol/mmol	5 months	<i>Dimorphotheca pluvialis</i>	C4	Herbaceous	Angio	350	650	umol/mol	86	UV			2.45	0.504	2.88	0.604	0.00	Photosynthesis	umol/m <sup>2</sup> /s	17.7	0.3404	26.50	5.404													
Ward et al., 1996	South Africa	OTC	ITE	umol/mmol	5 months	<i>Dimorphotheca pluvialis</i>	C4	Herbaceous	Angio	350	650	umol/mol	86	UV			2.27	0.444	3.53	0.524	0.00	Photosynthesis	umol/m <sup>2</sup> /s	22.404	6.04	27.304	6.04													
Wall et al., 2001	AZ, USA	FACE	WUE	umol/mol	2 growing seasons	<i>Sorghum bicolor</i>	C4	Herbaceous	Angio	A	A+200	umol/mol	53	H <sub>2</sub> O			84.0	10.33	132.0	10.33	0.00	Photosynthesis	umol/m <sup>2</sup> /s	38.794	353	42.094	353		St cond	mol/m <sup>2</sup> /s		0.59	0.09	3	0.37	0.09	3			
Vu and Allen, 2009b	FL, USA	GH	WUE	mol/mol	4 months	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	umol/mol	100	Temperature			104.0	25.48	203.0	69.48	0.00	Photosynthesis	umol/m <sup>2</sup> /s	36.50	14.78	38.00	12.48		St cond	mmol/m <sup>2</sup> /s		352.0	138.8	0	187.0	96.1	78			
Vu and Allen, 2009b	FL, USA	GH	WUE	mol/mol	4 months	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	umol/mol	100	Temperature			111.0	33.98	182.0	45.28	0.00	Photosynthesis	umol/m <sup>2</sup> /s	34.80	10.48	35.60	11.08		St cond	mmol/m <sup>2</sup> /s		314.0	121.68	0	196.0	96.1	78			
Vu and Allen, 2009b	FL, USA	GH	WUE	mol/mol	4 months	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	umol/mol	100	Temperature			112.0	33.98	209.0	62.28	0.00	Photosynthesis	umol/m <sup>2</sup> /s	36.80	12.78	37.20	0.058		St cond	mmol/m <sup>2</sup> /s		330.0	115.98	0	178.0	93.3	48			
Vu and Allen, 2009b	FL, USA	GH	WUE	mol/mol	4 months	<i>Saccharum officinarum</i>	C4	Crop	Angio	360	720	umol/mol	100	Temperature			115.0	48.08	176.0	98.00	0.00	Photosynthesis	umol/m <sup>2</sup> /s	34.50	16.98	37.00	11.68		St cond	mmol/m <sup>2</sup> /s		330.0	113.8	0	210.0	76.3	8			
Sebastiani et al., 2002	Italy	FACE	ITE	umol/mol	5 months	<i>Olea europaea</i>	C3	Woody	Angio	360	560	umol/mol	100				8.28	1.728	13.44	9.88	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.68	1.468	26.85	5.478													
Sebastiani et al., 2002	Italy	FACE	ITE	umol/mol	5 months	<i>Olea europaea</i>	C3	Woody	Angio	360	560	umol/mol	100				9.27	1.738	12.82	10.8	0.00	Photosynthesis	umol/m <sup>2</sup> /s	15.30	1.468	23.44	5.838													
Ghasemzadeh and Jaafar, 2011	Malaysia	GC	ITE	umol/mol	16 weeks	<i>Zingiber officinale</i>	C3	Herbaceous	Angio	400	800	umol/mol	100				1.52	0.089	1.85	0.049	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.58	0.249	9.22	0.359													
Ghasemzadeh and Jaafar, 2011	Malaysia	GC	ITE	umol/mol	16 weeks	<i>Zingiber officinale</i>	C3	Herbaceous	Angio	400	800	umol/mol	100				0.99	0.049	1.48	0.019	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.86	0.039	10.09	0.149													
Qaderi et al., 2005	Canada	GC	ITE	umol/mmol	1 month X 2	<i>Brassica napus</i>	C4	Crop	Angio	370	740	umol/mol	100	UV			2.64	0.186	4.94	0.126	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.14	0.226	3.13	0.206		Transpiration	mmol/m <sup>2</sup> /s		0.81	0.05	6	0.63	0.02	6			
Qaderi et al., 2005	Canada	GC	ITE	umol/mmol	1 month X 2	<i>Brassica napus</i>	C4	Crop	Angio	370	740	umol/mol	100	UV			2.17	0.226	3.43	0.176	0.00	Photosynthesis	umol/m <sup>2</sup> /s	1.82	0.106	2.35	0.126		Transpiration	mmol/m <sup>2</sup> /s		0.63	0.02	6	0.84	0.07	6			
Prins et al., 2011	South Africa	GC	WUE	umol/mol	8 weeks	<i>Zea mays</i>	C4	Crop	Angio	350	700	uL/L	100	Leaf Rank			146.26	44.3	204.35	35.94	0.00	Photosynthesis	umol/m <sup>2</sup> /s	14.520	304	12.194	744		St cond	mmol/m <sup>2</sup> /s		100.9	17.634	8	58.82	15.634				
Prins et al., 2011	South Africa	GC	WUE	umol/mol	8 weeks	<i>Zea mays</i>	C4	Crop	Angio	350	700	uL/L	100	Leaf Rank			183.44	43.8	207.5	17.74	0.00	Photosynthesis	umol/m <sup>2</sup> /s	20.892	964	17.195	934		St cond	mmol/m <sup>2</sup> /s		118.633	334	3	83.32	27.444				
Possell and Hewitt, 2009	UK	WTC	WUE	mmol/mol	120 days	<i>Acacia nigrescens</i>	C3	Woody	Angio	386	597	uL/L	55				0.11	0.033	0.13	0.023	0.00	Photosynthesis	umol/m <sup>2</sup> /s	9.17	1.993	10.24	1.323		St cond	mmol/m <sup>2</sup> /s		104.3	37.983		82.1	19.49	3			
Novriyanti et al., 2012	Indonesia	GC	ITE	mmol/mol	5 months	<i>Eucalyptus urophylla</i>	C3	Woody	Angio	380	760	umol/mol	100	Nitrogen			4.04	0.823	5.10	2.573	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.03	1.183	6.71	3.293		Transpiration	mmol/m <sup>2</sup> /s		0.70	0.23	3	1.64	1.08	3			
Novriyanti et al., 2012	Indonesia	GC	ITE	mmol/mol	5 months	<i>Eucalyptus urophylla</i>	C3	Woody	Angio	380	760	umol/mol	100	Nitrogen			6.79	0.993	9.84	0.743	0.00	Photosynthesis	umol/m <sup>2</sup> /s	20.05	7.93	31.71	1.053		Transpiration	mmol/m <sup>2</sup> /s		3.06	1.12	3	3.26	0.31	3			
Novriyanti et al., 2012	Indonesia	GC	ITE	mmol/mol	5 months	<i>Eucalyptus deglupta X Eucalyptus camadulensis</i>	C3	Woody	Angio	380	760	umol/mol	100	Nitrogen			2.38	0.503	6.30	2.683	0.00	Photosynthesis	umol/m <sup>2</sup> /s	9.47	3.293	11.18	1.973		Transpiration	mmol/m <sup>2</sup> /s		4.12	1.06	3	2.00	0.71	3			
Novriyanti et al., 2012	Indonesia	GC	ITE	mmol/mol	5 months	<i>Eucalyptus deglupta X Eucalyptus camadulensis</i>	C3	Woody	Angio	380	760	umol/mol	100	Nitrogen			4.75	0.933	9.10	1.223	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.74	7.43	32.76	1.183		Transpiration	mmol/m <sup>2</sup> /s		3.54	0.45	3	3.68	0.68	3			
Liet al., 2013	China	CTC	WUE	umol/mol	3 months	<i>Glycine max</i>	C3	Crop	Angio	380	740	umol/mol	95	H <sub>2</sub> O			47.1	1.575	76.4	28.25	0.00	Photosynthesis	umol/m <sup>2</sup> /s	9.88	0.835	12.10	1.005		St cond	mol/m <sup>2</sup> /s		0.21	0.01	5	0.17	0.02	5			
Liet al., 2013	China	CTC	ITE	umol/mol	3 months	<i>Glycine max</i>	C3	Crop	Angio	380	740	umol/mol	95	H <sub>2</sub> O			2.88	0.445	4.62	0.185	0.00	Photosynthesis	umol/m <sup>2</sup> /s	9.88	0.835	12.10	1.005		Transpiration	mmol/m <sup>2</sup> /s		3.42	0.17	5	2.60	0.17	5			

References	Location	Facility	Parameter X	Units X	Duration	Species	Control C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XNE	XSE	XNE	Parameter Y	Units Y	YA	YSA	YNA	YNE	YSE	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZNE	ZSE	ZNE
												mol											/s														
Liet al., 2013	China	CTC	WUE	kg/m <sup>3</sup>	3 months	<i>Glycine max</i>	C3	Crop	Angio	380	740	umol/mol	95	H <sub>2</sub> O		3.33	0.775	5.18	0.465	0.00	Shoot biomass	g	3.92	0.765	5.23	1.925			Evapotrans	mm	185.8	11.85	0	107.6	14.05		
Haworth et al., 2011	Canada	GC	ITE	mmol/mol	18 months	<i>Agathus australis</i>	C3	Woody	Angio	380	1500	ppm	295			6.52	0.903	30.26	6.33	0.00	Photosynthesis	umol/m <sup>2</sup> /s	7.23	0.473	19.50	0.713											
Haworth et al., 2011	Canada	GC	ITE	mmol/mol	18 months	<i>Araucana bidwillii</i>	C3	Woody	Angio	380	1500	ppm	295			6.26	0.903	30.78	10.33	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.68	0.943	2.32	0.473											
Haworth et al., 2011	Canada	GC	ITE	mmol/mol	18 months	<i>Wolemia nobilis</i>	C3	Woody	Angio	380	1500	ppm	295			5.74	0.483	18.52	1.363	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.05	0.943	2.59	0.713											
Gessler et al., 2009	Germany	OTC	ITE	umol/mmol	4 weeks	<i>Aster tripolium</i>	C3	Herbaceous	Angio	380	520	ppm	37			5.10	1.60	12.58	1.50	12.00	Photosynthesis	umol/m <sup>2</sup> /s	21.65	10.12	93.60	6.30	12		Transpiration	mol/m <sup>2</sup> /s	4.30	0.90	12	6.10	1.50	12	
Gessler et al., 2009	Germany	OTC	ITE	umol/mmol	4 weeks	<i>Aster tripolium</i>	C3	Herbaceous	Angio	380	520	ppm	37			4.30	0.90	12.54	1.10	12.00	Photosynthesis	umol/m <sup>2</sup> /s	11.45	10.12	20.80	7.90	12		Transpiration	mol/m <sup>2</sup> /s	2.80	1.40	12	4.00	1.90	12	
Gessler et al., 2009	Germany	OTC	ITE	umol/mmol	4 weeks	<i>Aster tripolium</i>	C3	Herbaceous	Angio	380	520	ppm	37			3.50	0.40	12.56	0.30	12.00	Photosynthesis	umol/m <sup>2</sup> /s	7.60	4.60	12.13	0.480	12		Transpiration	mol/m <sup>2</sup> /s	2.30	1.60	12	2.50	1.30	12	
Diemer, 1994	Switzerland	OTC	ITE	umol/mmol	4 weeks	<i>Carex curvula</i>	C4	Herbaceous	Angio	257	528	ubar/bar	105	Nutrient		2.30	0.69	12.24	2.08	12.00	Photosynthesis	umol/m <sup>2</sup> /s	9.63	3.34	12.13	4.93	12		Evapotrans	mmol/m <sup>2</sup> /s	4.19	1.03	12	3.10	1.03	12	
Diemer, 1994	Switzerland	OTC	ITE	umol/mmol	4 weeks	<i>Carex curvula</i>	C4	Herbaceous	Angio	257	528	ubar/bar	105	Nutrient		3.10	1.00	5.00	1.20	4.00	Photosynthesis	umol/m <sup>2</sup> /s	13.74	0.824	24.59	2.474			Evapotrans	mmol/m <sup>2</sup> /s	4.40	1.78	4	5.20	2.79	4	
Choi et al., 2005	Japan	GC	ITE	umol/mmol	18 weeks	<i>Pinus densiflora</i>	C3	Woody	Gymno	360	720	umol/mol	100	Fungus		31.32	4.13	9	21.64	4.33	3.00	Photosynthesis	umol/m <sup>2</sup> /s	7.86	2.373	6.55	1.283										
Choi et al., 2005	Japan	GC	ITE	umol/mmol	18 weeks	<i>Pinus densiflora</i>	C3	Woody	Gymno	360	720	umol/mol	100	Fungus		31.10	0.963	32.50	0.963	3.00	Photosynthesis	umol/m <sup>2</sup> /s	11.78	1.133	13.22	3.343											
Wang et al., 2012	China	OTC	WUE	mmol/mol	40 days	<i>Populus euramericana</i>	C3	Woody	Angio	360	800	umol/mol	122			36.49	9.13	1	138.86	43.00	Photosynthesis	umol/m <sup>2</sup> /s	23.18	1.213	33.13	10.13			St cond	mol/m <sup>2</sup> /s	0.64	0.12	3	0.26	0.17	3	
Wang et al., 2012	China	OTC	WUE	mmol/mol	40 days	<i>Salix jianguensis</i>	C3	Woody	Angio	360	800	umol/mol	122			59.42	27.33	6	195.81	101.30	Photosynthesis	umol/m <sup>2</sup> /s	23.83	0.233	27.96	6.673			St cond	mol/m <sup>2</sup> /s	0.42	0.19	3	0.14	0.05	3	
Wang et al., 2012	China	OTC	WUE	mmol/mol	40 days	<i>Salix jianguensis</i>	C3	Woody	Angio	360	800	umol/mol	122			46.51	13.83	6	156.44	40.33	Photosynthesis	umol/m <sup>2</sup> /s	23.03	1.063	39.19	6.773			St cond	mol/m <sup>2</sup> /s	0.51	0.16	3	0.26	0.12	3	
Thomas et al., 1994	NC, USA	GH	ITE	umol/mmol	115 days	<i>Pinus taeda</i>	C3	Woody	Gymno	35	65	Pa	86	Nutrient		3.50	0.489	6.44	0.849	9.00	Photosynthesis	umol/m <sup>2</sup> /s	5.21	0.519	8.31	1.509											
Thomas et al., 1994	NC, USA	GH	ITE	umol/mmol	115 days	<i>Pinus taeda</i>	C3	Woody	Gymno	35	65	Pa	86	Nutrient		3.26	0.819	3.98	0.849	9.00	Photosynthesis	umol/m <sup>2</sup> /s	4.13	0.729	4.71	2.109											
Thomas et al., 1994	NC, USA	GH	ITE	umol/mmol	115 days	<i>Pinus taeda</i>	C3	Woody	Gymno	35	65	Pa	86	Nutrient		2.50	0.699	3.10	0.689	9.00	Photosynthesis	umol/m <sup>2</sup> /s	3.75	0.579	3.70	0.369											
Szente eta l., 1998	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	231 days	<i>Dactylis glomerata</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			0.58	0.487	1.01	0.887	7.00	Photosynthesis	umol/m <sup>2</sup> /s	1.63	1.167	5.21	5.007			transpiration	umol/m <sup>2</sup> /s	2.97	0.66	7	5.06	1.01	7	
Szente eta l., 1998	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	231 days	<i>Festuca rupicola</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			0.77	0.297	1.23	0.487	7.00	Photosynthesis	umol/m <sup>2</sup> /s	4.64	1.887	7.06	2.627			transpiration	umol/m <sup>2</sup> /s	6.03	1.32	7	5.76	0.53	7	
Szente eta l., 1998	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	231 days	<i>Filipendula vulgaris</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			0.29	0.117	1.26	0.667	7.00	Photosynthesis	umol/m <sup>2</sup> /s	1.61	0.497	8.41	2.757			transpiration	umol/m <sup>2</sup> /s	5.55	1.08	7	6.74	1.43	7	
Szente eta l., 1998	Hungary	OTC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	231 days	<i>Salvia nemorosa</i>	C3	Herbaceous	Angio	350	700	umol/mol	100			0.32	0.247	1.59	0.137	7.00	Photosynthesis	umol/m <sup>2</sup> /s	1.87	0.987	12.13	1.677			transpiration	umol/m <sup>2</sup> /s	6.62	4.10	7	7.63	0.69	7	
Mark and Tevni., 1996	Portugal	GC	ITE	mol/mol	18 days	<i>Helianthus annuus</i>	C4	Crop	Angio	340	680	uL/L	100	UV		7.54	2.624	10.03	3.194	0.00	Photosynthesis	umol/plant/s	50.647	97.40	72.30	9.6340			transpiration	umol/plant/s	7.16	1.56	40	6.87	2.27	40	
Mark and Tevni., 1996	Portugal	GC	ITE	mol/mol	18 days	<i>Zea mays</i>	C4	Crop	Angio	340	680	uL/L	100	UV		6.56	1.824	14.74	5.984	0.00	Photosynthesis	umol/plant/s	70.53	64.40	68.38	13.940			transpiration	umol/plant/s	11.38	3.02	40	4.90	2.04	40	
Liet al., 2010	MD, USA	OTC	ITE	umol/mmol	12 years	<i>Scirpus olneyi</i>	C3	Herbaceous	Angio	Ambient	A + 340	umol/mol	89			3.36	0.521	5.98	1.561	10.00	Canopy photo	umol/m <sup>2</sup> /s	26.547	30.10	36.19	10.910			Evapotrans	mmol/m <sup>2</sup> /s	7.95	1.04	10	6.31	0.78	10	
Liet al., 2010	MD, USA	OTC	ITE	umol/mmol	12 years	<i>Spartina patens</i>	C4	Herbaceous	Angio	Ambient	A + 341	umol/mol	89			3.28	0.521	5.98	2.071	10.00	Canopy photo	umol/m <sup>2</sup> /s	20.74	4.8710	23.46	0.0810			Evapotrans	mmol/m <sup>2</sup> /s	6.72	1.30	10	4.43	0.78	10	
Kubiske and Pregitzer, 1997	MI, USA	OTC	ITE	mmol/mol	90 days	<i>Betula papyrifera</i>	C3	Woody	Angio	Ambient	700	ppm	84	Shade		1.53	0.293	1.77	0.233	0.00	Photosynthesis	umol/m <sup>2</sup> /s	6.51	1.283	12.92	1.613											
Kubiske and Pregitzer, 1997	MI, USA	OTC	ITE	mmol/mol	90 days	<i>Betula papyrifera</i>	C3	Woody	Angio	Ambient	700	ppm	84	Shade		0.98	0.163	1.28	0.143	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.69	0.483	6.64	0.593											
Kubiske and Pregitzer, 1997	MI, USA	OTC	ITE	mmol/mol	90 days	<i>Acer rubrum</i>	C3	Woody	Angio	Ambient	700	ppm	84	Shade		1.32	0.383	1.14	0.383	0.00	Photosynthesis	umol/m <sup>2</sup> /s	1.79	0.363	2.98	0.833											
Kubiske and Pregitzer, 1997	MI, USA	OTC	ITE	mmol/mol	90 days	<i>Acer rubrum</i>	C3	Woody	Angio	Ambient	700	ppm	84	Shade		0.91	0.093	1.38	0.233	0.00	Photosynthesis	umol/m <sup>2</sup> /s	2.91	0.383	4.87	0.733											
Kus et al., 2001	MI, USA	OTC	ITE	umol/mmol	127 days	<i>Plantago lanceolata</i>	C3	Herbaceous	Angio	35	71	Pa	103			1.78	0.4789	2.76	0.9489	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.004	7.289	19.59	7.5589			Transpiration	mmol/m <sup>2</sup> /s	7.29	2.08	89	7.16		89	

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XNE	XSE	XNE	Parameter Y	Units Y	YA	YSA	YNA	YNE	YSE	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZNE	ZSE	ZNE	
Keutgen and Chen, 2001	Germany	OTC	ITE	umol/mmol	90 days	<i>Citrus madurensis</i>	C3	Woody	Angio	450	750	ppm	67				3.20	0.305	2.40	1.305	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.30	2.305	2.40	1.505			Transpiration	mmol/m <sup>2</sup> /s	2.00	0.70	5	1.00	0.40	5	
Keutgen and Chen, 2001	Germany	OTC	ITE	umol/mmol	90 days	<i>Citrus madurensis</i>	C3	Woody	Angio	600	900	ppm	60				4.30	1.205	5.90	2.305	0.00	Photosynthesis	umol/m <sup>2</sup> /s	6.10	2.105	2.40	1.105			Transpiration	mmol/m <sup>2</sup> /s	1.60	0.90	5	0.50	0.30	5	
Hirano et al., 2012	Japan	GC	ITE	umol/mmol	2 months	<i>Fagus crenata</i>	C3	Woody	Angio	200	550	uL/L	175				1.80	0.20	4.70	0.84	10.00	Photosynthesis	umol/m <sup>2</sup> /s	2.90	0.30	4.30	0.70	10		Transpiration	mmol/m <sup>2</sup> /s	1.62	0.19	10	0.87	0.16	10	
Hirano et al., 2012	Japan	GC	ITE	umol/mmol	2 months	<i>Fagus crenata</i>	C3	Woody	Angio	350	750	uL/L	114				3.60	0.41	10	7.50	1.83	10.00	Photosynthesis	umol/m <sup>2</sup> /s	4.30	0.40	5.10	0.70	10		Transpiration	mmol/m <sup>2</sup> /s	1.13	0.21	10	0.72	0.15	10
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	250	430	uL/L	72	Soil type			1.56	4.03	48	2.85	1.39	48.00	Photosynthesis	umol/m <sup>2</sup> /s	6.42	10.04	7.62	7.51	48									
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	350	500	uL/L	43	Soil type			2.19	2.63	48	3.40	3.79	48.00	Photosynthesis	umol/m <sup>2</sup> /s	7.06	6.68	48	8.39	9.74	48								
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	250	430	uL/L	72	Soil type			0.84	2.79	48	2.68	2.71	48.00	Photosynthesis	umol/m <sup>2</sup> /s	3.69	7.79	48	8.51	7.23	48								
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	350	500	uL/L	43	Soil type			1.77	1.78	48	3.56	4.93	48.00	Photosynthesis	umol/m <sup>2</sup> /s	6.18	4.17	48	10.84	11.64	48								
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	250	430	uL/L	72	Soil type			0.68	1.94	48	2.85	2.17	48.00	Photosynthesis	umol/m <sup>2</sup> /s	3.73	7.51	48	7.30	5.01	48								
Fay et al., 2009	TX, USA	LYCO G	ITE	umol/mol	1 year	<i>Solidago canadensis</i>	C3	Herbaceous	Angio	350	500	uL/L	43	Soil type			1.77	1.78	48	3.92	3.29	48.00	Photosynthesis	umol/m <sup>2</sup> /s	5.45	5.01	48	9.11	7.23	48								
Danyaghi and Dang, 2013	Canada	GH	ITE	umol/mol	1 growing season	<i>Acer spicatum</i>	C3	Woody	Angio	392	784	umol/mol	100	Shade			3.34	0.89	10	5.18	1.03	10.00	Photosynthesis	umol/m <sup>2</sup> /s	5.27	0.81	10	5.42	1.04	10								
Danyaghi and Dand, 2013	Canada	GH	ITE	umol/mol	1 growing season	<i>Acer spicatum</i>	C3	Woody	Angio	392	784	umol/mol	100	Shade			3.59	0.84	10	7.80	1.59	10.00	Photosynthesis	umol/m <sup>2</sup> /s	4.95	1.16	10	6.96	1.16	10								
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Albizia adnacephala</i>	C3	Woody	Angio	40	70	Pa	75				2.30	0.195	3.58	0.895	0.00	Biomass	g	19.83	3.635	22.73	2.265			Water use	kg/plant	7.73	1.61	5	5.77	1.84	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Dalbergia retusa</i>	C3	Woody	Angio	40	70	Pa	75				2.18	0.545	3.16	0.735	0.00	Biomass	g	49.32	9.75	48.38	4.15			Water use	kg/plant	20.72	3.22	5	14.23	7.15	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Inga punctata</i>	C3	Woody	Angio	40	70	Pa	75				1.78	0.215	2.84	0.395	0.00	Biomass	g	11.38	4.385	13.64	6.535			Water use	kg/plant	5.67	1.61	5	4.12	1.84	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Omosia macrocalyx</i>	C3	Woody	Angio	40	70	Pa	75				2.36	0.195	4.12	0.545	0.00	Biomass	g	16.56	8.15	38.31	16.75			Water use	kg/plant	6.19	3.00	5	7.94	3.46	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Schizobolium parahyba</i>	C3	Woody	Angio	40	70	Pa	75				2.47	0.215	4.57	0.425	0.00	Biomass	g	58.77	7.265	53.90	7.265			Water use	kg/plant	21.34	2.77	5	10.62	1.38	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Chrysophyllum cainito</i>	C3	Woody	Angio	40	70	Pa	75				1.61	0.195	2.13	0.285	0.00	Biomass	g	8.77	2.905	10.395	8.15			Water use	kg/plant	4.54	1.38	5	4.02	2.07	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Coccoloba uvifera</i>	C3	Woody	Angio	40	70	Pa	75				2.18	0.405	3.30	0.395	0.00	Biomass	g	26.62	5.535	33.77	13.05			Water use	kg/plant	10.00	2.07	5	8.35	3.23	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Hieronyma alchomeddes</i>	C3	Woody	Angio	40	70	Pa	75				2.06	0.145	3.13	0.395	0.00	Biomass	g	36.36	7.995	46.43	8.715			Water use	kg/plant	15.13	3.00	5	12.89	2.54	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Pachira quinata</i>	C3	Woody	Angio	40	70	Pa	75				2.17	0.075	2.99	0.475	0.00	Biomass	g	74.33	10.15	90.28	15.95			Water use	kg/plant	29.28	4.38	5	25.63	4.46	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	3 months	<i>Swietenia macrophylla</i>	C3	Woody	Angio	40	70	Pa	75				1.58	0.215	2.31	0.635	0.00	Biomass	g	7.47	2.185	9.09	6.085			Water use	kg/plant	4.02	1.61	5	3.30	2.31	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	109 days	<i>Swietenia macrophylla</i>	C3	Woody	Angio	40	70	Pa	75	H <sub>2</sub> O	Nutrients		2.61	0.345	3.82	0.415	0.00	Biomass	g	48.09	18.35	84.70	23.25			Water use	kg/plant	16.48	4.60	5	19.74	4.86	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	109 days	<i>Swietenia macrophylla</i>	C3	Woody	Angio	40	70	Pa	75	H <sub>2</sub> O	Nutrients		1.61	0.205	2.33	0.615	0.00	Biomass	g	7.65	1.225	9.29	4.895			Water use	kg/plant	4.11	1.28	5	3.31	2.30	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	109 days	<i>Omosia macrocalyx</i>	C3	Woody	Angio	40	70	Pa	75	H <sub>2</sub> O	Nutrients		2.61	0.075	4.52	0.345	0.00	Biomass	g	24.04	5.555	56.83	21.95			Water use	kg/plant	8.11	3.07	5	11.04	4.86	5	
Cemusak et al., 2011	Panama	GH	WUE	g/Kg	109 days	<i>Omosia macrocalyx</i>	C3	Woody	Angio	40	70	Pa	75	H <sub>2</sub> O	Nutrients		2.36	0.205	4.12	0.545	0.00	Biomass	g	16.39	5.115	38.22	15.85			Water use	kg/plant	6.17	3.07	5	7.89	3.58	5	
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis flexuosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		1.80	0.386	1.65	0.116	0.00	Photosynthesis	umol/m <sup>2</sup> /s	18.83	3.086	11.35	8.76											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis flexuosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		1.52	0.386	2.86	1.536	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.03	4.696	13.72	3.966											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis flexuosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		1.91	0.386	3.07	1.076	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.72	2.206	18.14	4.266											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis flexuosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		2.42	0.786	3.62	0.146	0.00	Photosynthesis	umol/m <sup>2</sup> /s	14.58	4.706	18.34	4.846											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis glandulosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		1.99	0.606	2.17	1.566	0.00	Photosynthesis	umol/m <sup>2</sup> /s	15.72	2.206	12.60	10.26											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis glandulosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		1.77	0.436	3.19	0.966	0.00	Photosynthesis	umol/m <sup>2</sup> /s	18.12	2.646	14.98	3.366											
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis glandulosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio		2.38	0.486	3.58	0.466	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.62	2.206	18.03	3.236											



References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XEA	XNE	Parameter Y	Units Y	YA	YSA	YNA	YEA	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZEA	ZNE		
Causin et al., 2006	Argentina	GC	ITE	umol/mmol	39 days	<i>Prosopis glandulosa</i>	C3	Woody	Angio	350	650	ppm	86	Nitrogen	Nitrate ratio	2.17	0.606	4.62	1.486	0.00	Photosynthesis	umol/m <sup>2</sup> /s	15.37	1.476	19.482	6.645										
Qaden and Reid, 2008	Canada	GC	ITE	umol/mmol	40 days	<i>Siene noctiflora</i>	C4	Herbaceous	Angio	370	740	umol/mol	100	Temperature		1.08	1.569	0.49	0.899	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.37	1.569	2.80	0.899		Transpiration	mmol/m <sup>2</sup> /s	3.52	1.56	9	6.06	0.89	9	
Qaden and Reid, 2008	Canada	GC	ITE	umol/mmol	40 days	<i>Siene noctiflora</i>	C4	Herbaceous	Angio	370	740	umol/mol	100	Temperature		0.90	1.179	1.51	1.179	0.00	Photosynthesis	umol/m <sup>2</sup> /s	3.57	1.179	4.13	1.179		Transpiration	mmol/m <sup>2</sup> /s	5.32	1.17	9	2.77	1.17	9	
LeThiec and Dixon, 1996	France	OTC	WUE	umol/mol	180 days	<i>Picea abies</i>	C3	Woody	Gymno	350	740	umol/mol	111			69.1	18.408	112.2	38.78	0.00																
LeThiec and Dixon, 1996	France	OTC	WUE	umol/mol	180 days	<i>Quercus rubra</i>	C3	Woody	Angio	350	740	umol/mol	111			94.0	37.080	133.6	42.18	0.00																
Zhou et al., 2013	China	OTC	ITE	umol/mmol	10 years	<i>Pinus sylvestrifomis</i>	C3	Woody	Gymno	370	500	umol/mol	85			3.90	0.693	5.40	0.873	0.00	Photosynthesis	umol/m <sup>2</sup> /s	11.00	1.563	11.702	2.253		Transpiration	mmol/m <sup>2</sup> /s	3.10	0.69	3	2.60	1.04	3	
Zhou et al., 2013	China	OTC	ITE	umol/mmol	10 years	<i>Pinus koraiensis</i>	C3	Woody	Gymno	370	500	umol/mol	85			4.20	1.043	5.90	0.873	0.00	Photosynthesis	umol/m <sup>2</sup> /s	8.20	1.213	11.201	9.13		Transpiration	mmol/m <sup>2</sup> /s	2.40	0.69	3	2.20	0.69	3	
Carter et al., 1997	UK	GC	WUE	g/Kg	9 weeks	<i>Lotus comiculatus</i>	C3	Herbaceous	Angio	350	700	ppm	100	H <sub>2</sub> O	Temperature	2.37	0.5915	3.52	0.4215	0.00	Shoot biomass	g	55.20	19.915	54.70	18.515										
Carter et al., 1997	UK	GC	WUE	g/Kg	9 weeks	<i>Lotus comiculatus</i>	C3	Herbaceous	Angio	350	700	ppm	100	H <sub>2</sub> O	Temperature	1.85	0.3415	4.17	0.8415	0.00	Shoot biomass	g	29.70	6.8515	36.52	6.8515										
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	3 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	Ambient	Ambient+350	umol/mol	92			0.02	0.003	0.05	0.023	0.00	Photosynthesis	umol/m <sup>2</sup> /s	24.821	6.53	37.033	2.83		St cond	mmol/m <sup>2</sup> /s	1184	306.63	774	4281	43		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	3 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	Ambient	Ambient+351	umol/mol	92			0.03	0.013	0.07	0.013	0.00	Photosynthesis	umol/m <sup>2</sup> /s	25.94	7.33	32.2	6.443		St cond	mmol/m <sup>2</sup> /s	946.0	465.63	472.2	3128	83		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	3 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	Ambient	Ambient+352	umol/mol	92			0.04	0.013	0.08	0.013	0.00	Photosynthesis	umol/m <sup>2</sup> /s	25.694	0.53	37.284	2.73		St cond	mmol/m <sup>2</sup> /s	615.1	167.43	467.2	323	243		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	3 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	Ambient	Ambient+353	umol/mol	92			0.04	0.013	0.11	0.033	0.00	Photosynthesis	umol/m <sup>2</sup> /s	16.302	4.93	23.705	5.553		St cond	mmol/m <sup>2</sup> /s	420.4	158.43	252.3	127	83		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	3 years	<i>Solanum tuberosum</i>	C3	Crop	Angio	Ambient	Ambient+353	umol/mol	92			0.05	0.013	0.12	0.023	0.00	Photosynthesis	umol/m <sup>2</sup> /s	17.862	8.53	28.262	4.33		St cond	mmol/m <sup>2</sup> /s	385.5	135.33	248.3	19	0.33		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	2 years	<i>Sorghum bicolor</i>	C4	Herbaceous	Angio	Ambient	Ambient+354	umol/mol	92			0.06	0.014	0.12	0.034	0.00	Photosynthesis	umol/m <sup>2</sup> /s	54.074	1.14	54.074	1.14		St cond	mmol/m <sup>2</sup> /s	912.3	170.94	555.2	124	84		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	2 years	<i>Sorghum bicolor</i>	C4	Herbaceous	Angio	Ambient	Ambient+355	umol/mol	92			0.10	0.024	0.20	0.034	0.00	Photosynthesis	umol/m <sup>2</sup> /s	41.31	1.544	47.2	4.444		St cond	mmol/m <sup>2</sup> /s	409.9	67.904	246.5	4	234		
Bunce, 2003	MD, USA	OTC	WUE	umol/mmol	2 years	<i>Sorghum bicolor</i>	C4	Herbaceous	Angio	Ambient	Ambient+356	umol/mol	92			0.12	0.033	0.27	0.033	0.00	Photosynthesis	umol/m <sup>2</sup> /s	38.943	3.83	44.5	0.683		St cond	mmol/m <sup>2</sup> /s	339.2	69.793	169.2	14	2.3		
Guehl et al., 1994	France	GH	WUE	10-3 g/g	7 months	<i>Pinus pinaster</i>	C3	Woody	Gymno	350	700	umol/mol	100	H <sub>2</sub> O		4.92	0.4710	7.31	0.4710	0.00	biomass	g/plant	11.52	2.0810	19.474	1710		Water use	g/plant	2892	546.010	3237	819	010		
Guehl et al., 1994	France	GH	WUE	10-3 g/g	7 months	<i>Quercus petraea</i>	C3	Woody	Angio	350	700	umol/mol	100	H <sub>2</sub> O		7.05	0.4710	12.36	0.4710	0.00	biomass	g/plant	11.463	6510	27.277	8110		Water use	g/plant	4187	109210	5784	1365	10		
Atkinson et al., 1997	France	GH	ITE	umol/mmol	10 months	<i>Quercus robur</i>	C3	Woody	Angio	350	700	ppm	100	Fungus		5.08	0.7910	12.43	0.7910	0.00	Photosynthesis	umol/m <sup>2</sup> /s	5.50	1.3510	14.201	3510		Transpiration	mmol/m <sup>2</sup> /s	1.08	0.16	10	1.14	0.16	10	
Atkinson et al., 1997	France	GH	ITE	umol/mmol	10 months	<i>Prunus avium X pseudocerasus</i>	C3	Woody	Angio	350	700	ppm	100			5.09	0.7810	8.48	0.7810	0.00	Photosynthesis	umol/m <sup>2</sup> /s	6.60	1.2210	5.30	1.2210		Transpiration	mmol/m <sup>2</sup> /s	1.28	0.14	10	0.65	0.14	10	
Coleman and Bazzaz, 1992	IL, USA	GC	WUE	umol/mol	60 days	<i>Abutilon theophrasti</i>	C3	Herbaceous	Angio	400	700	uL/L	75	Temperature		72.6	12.814	122.7	16.414	0.00	Photosynthesis	umol/m <sup>2</sup> /s	12.373	6.714	18.073	3214		St cond	mol/m <sup>2</sup> /s	0.18	0.06	14	0.15	0.03	14	
Coleman and Bazzaz, 1992	IL, USA	GC	WUE	umol/mol	60 days	<i>Abutilon theophrasti</i>	C3	Herbaceous	Angio	400	700	uL/L	75	Temperature		57.3	18.019	102.9	43.519	0.00	Photosynthesis	umol/m <sup>2</sup> /s	12.634	9.119	15.096	7419		St cond	mol/m <sup>2</sup> /s	0.24	0.11	19	0.16	0.08	19	
Coleman and Bazzaz, 1992	IL, USA	GC	WUE	umol/mol	60 days	<i>Amaranthus retroflexus</i>	C4	Herbaceous	Angio	400	700	uL/L	75	Temperature		104	27.117	152.6	68.919	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.4	3.1517	14.3	5.1719		St cond	mol/m <sup>2</sup> /s	0.13	0.03	17	0.11	0.03	19	
Coleman and Bazzaz, 1992	IL, USA	GC	WUE	umol/mol	60 days	<i>Amaranthus retroflexus</i>	C4	Herbaceous	Angio	400	700	uL/L	75	Temperature		76.4	30.414	118.6	67.316	0.00	Photosynthesis	umol/m <sup>2</sup> /s	13.64	8.214	13.267	7.916		St cond	mol/m <sup>2</sup> /s	0.20	0.09	14	0.12	0.06	16	
Leavitt et al., 2003	AR, USA	OTC	WUE	mmol/mol	8 years	<i>Citrus aurantium</i>	C3	Woody	Angio	400	700	umol/mol	75			87.57	5.045	158.6	7.94.00																	
Townend, 1993	UK	GH	ITE	umol/mmol	37 days	<i>Picea sitchensis</i>	C3	Woody	Gymno	350	600	ppm	71	H <sub>2</sub> O		3.31	2.4924	5.95	4.2922	0.00	Photosynthesis	umol/m <sup>2</sup> /s	4.54	3.3424	7.20	5.6422		Transpiration	mmol/m <sup>2</sup> /s	1.28	0.86	24	1.07	0.70	22	
Robinson, 1999	Australia	GC	WUE	g/g	41 days	<i>Panicum cobratum</i>	C4	Herbaceous	Angio	350	1000	uL/L	186	H <sub>2</sub> O		0.00	0.004	0.00	0.004	0.00	biomass	g/pot	4.56	4.074	5.31	4.704		Transpiration	g/pot	1687	11434	1111	659	44		
Igamberdiev et al., 2004	Denmark	GC	ITE	umol/mol	3 weeks	<i>Arabidopsis thaliana</i>	C3	Herbaceous	Angio	700	1400	uL/L	100			6.71	0.895	7.11	2.485	0.00																
Igamberdiev et al., 2004	Denmark	GC	ITE	umol/mol	3 weeks	<i>Arabidopsis thaliana</i>	C3	Herbaceous	Angio	700	1400	uL/L	100			4.62	1.595	7.02	2.395	0.00																

References	Location	Facility	Parameter X	Units X	Duration	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment 1	Interacting treatment 2	XA	XSA	XNA	XEA	XNE	Parameter Y	Units Y	YA	YSA	YNA	YEA	YNE	Parameter Z	Units Z	ZA	ZSA	ZNA	ZE	ZNE			
Tognetti et al., 2001	Italy	FACE	ITE	umol/mol	1 growing season	<i>Olea europaea</i>	C3	Woody	Angio	360	560	umol/mol	56			11.3	0.825	16.5	0.825	0.0	Photosynthesis	umol/m <sup>2</sup> /s	6.38	0.825	10.9	0.825											
Tognetti et al., 2001	Italy	FACE	ITE	umol/mol	1 growing season	<i>Olea europaea</i>	C3	Woody	Angio	360	560	umol/mol	56			13.1	0.825	17.2	0.825	0.0	Photosynthesis	umol/m <sup>2</sup> /s	7.03	0.825	13.5	0.825											
Gunderson et al., 2002	TN, USA	FACE	ITE	umol/mmol	3 growing seasons	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+200	umol/mol	63	Leaf rank		3.44	0.313	5.85	0.522	0.0	Photosynthesis	umol/m <sup>2</sup> /s	10.9	1.393	15.9	1.702											
Gunderson et al., 2002	TN, USA	FACE	ITE	umol/mmol	3 growing seasons	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+200	umol/mol	63	Leaf rank		3.56	0.573	6.33	0.822	0.0	Photosynthesis	umol/m <sup>2</sup> /s	9.00	2.773	13.1	2.832											
deAngelis macchia species.xls	Montalto di castro	OTC	WUE	umol/mol		<i>Pistacia lentiscus</i>	C3	Woody	Angio	360	700	umol/mol	94			54.8	19.823	72.7	50.2	24.0	Photosynthesis	umol/m <sup>2</sup> /s	5.64	3.06	23	6.21	3.10	24	St cond	mol/m <sup>2</sup> /s	0.10	0.04	23	0.14	0.16	24	
deAngelis macchia species.xls	Montalto di castro	OTC	WUE	umol/mol		<i>Quercus ilex</i>	C3	Woody	Angio	360	700	umol/mol	94			103.2	23.925	204.5	475.9	23.0	Photosynthesis	umol/m <sup>2</sup> /s	4.54	1.95	25	9.04	4.14	23	St cond	mol/m <sup>2</sup> /s	0.05	0.02	25	0.05	0.03	23	
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Achillea millefolium</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			42.2	4.104	89.7	61.8	4.0	Photosynthesis	umol/m <sup>2</sup> /s	13.3	4.404	15.6	6.024		St cond	mol/m <sup>2</sup> /s	0.32	0.11	4	0.23	0.13	4		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Agropyron repens</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			58.7	15.620	114.8	40.5	22.0	Photosynthesis	umol/m <sup>2</sup> /s	13.6	2.45	20	20.99	18.22	St cond	mol/m <sup>2</sup> /s	0.25	0.09	20	0.21	0.09	22		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Anemone cylindrica</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			54.8	16.93	88.2	32.7	8.0	Photosynthesis	umol/m <sup>2</sup> /s	12.0	1.253	18.7	5.145		St cond	mol/m <sup>2</sup> /s	0.23	0.06	3	0.24	0.10	5		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Bromus inermis</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			61.2	13.223	103.3	35.0	21.0	Photosynthesis	umol/m <sup>2</sup> /s	12.1	3.39	23	16.53	4.02	St cond	mol/m <sup>2</sup> /s	0.21	0.09	23	0.18	0.08	21		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Koeleria cristata</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			50.0	10.25	91.2	20.2	25.0	Photosynthesis	umol/m <sup>2</sup> /s	14.5	2.815	19.5	6.255		St cond	mol/m <sup>2</sup> /s	0.31	0.10	5	0.23	0.08	5		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Poa pratensis</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			62.0	5.683	162.3	61.8	4.0	Photosynthesis	umol/m <sup>2</sup> /s	6.24	2.273	17.0	5.384		St cond	mol/m <sup>2</sup> /s	0.10	0.04	3	0.11	0.03	4		
Crous et al., 2010	MN, USA	FACE	WUE	umol/mol	9 years	<i>Solidago rigida</i>	C3	Herbaceous	Angio	Ambient	560	umol/mol	47			71.3	25.08	75.2	36.2	11.0	Photosynthesis	umol/m <sup>2</sup> /s	18.7	5.318	21.6	4.01	11	St cond	mol/m <sup>2</sup> /s	0.31	0.18	8	0.34	0.14	11		
Elsworth D.	NC, USA	FACE	WUE	mmol/mol	8 years	<i>Pinus taeda</i>	C3	Woody	Gymno	Ambient	A+200	umol/mol	63	Nitrogen		81.7	27.488	116.8	32.1	70.0	Photosynthesis	umol/m <sup>2</sup> /s	12.0	4.87	88	17.2	4.91	70	St cond	mol/m <sup>2</sup> /s	0.16	0.07	88	0.16	0.07	70	
Elsworth D.	NC, USA	FACE	WUE	mmol/mol	8 years	<i>Pinus taeda</i>	C3	Woody	Gymno	Ambient	A+200	umol/mol	63	Nitrogen		84.1	24.447	125.4	43.3	44.0	Photosynthesis	umol/m <sup>2</sup> /s	10.0	2.65	47	14.7	2.91	44	St cond	mol/m <sup>2</sup> /s	0.13	0.05	47	0.13	0.05	44	
Elsworth D.	Australia	WTC	WUE	umol/mol	2 years	<i>Eucalyptus saligna</i>	C3	Woody	Angio	380	620	ppm	63	H <sub>2</sub> O		67.2	28.413	118.4	50.8	117.0	Photosynthesis	umol/m <sup>2</sup> /s	14.2	2.713	15.9	4.7	31	11	St cond	mol/m <sup>2</sup> /s	0.26	0.20	13	0.17	0.14	11	
Freeman 1998	Denmark	BB	WUE	umol/mol	2 years	<i>Fagus sylvatica</i>	C3	Woody	Angio	350	700	umol/mol	100			78.6	13.48	152.3	32.6	68.0	Photosynthesis	umol/m <sup>2</sup> /s	13.2	2.708	24.6	25.638		St cond	mol/m <sup>2</sup> /s	0.18	0.07	8	0.17	0.06	8		
Warren et al., 2011	TN, USA	FACE	WUE	umol/mol	3 growing seasons	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+200	umol/mol	63			61.7	15.273	92.0	23.2	74.0	Photosynthesis	umol/m <sup>2</sup> /s	7.67	3.68	73	10.1	5.22	74	St cond	mol/m <sup>2</sup> /s	0.14	0.09	73	0.12	0.08	74	
Kelly 2014	Australia	GH	WUE	g/liter	301 days	<i>Eucalyptus pilularis</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		5.5	0.486	8.78	0.746	0.0	biomass	g	1657	106	6	2578	257	6	Water use	liters	299	319	0	6	293	323	236
Kelly 2014	Australia	GH	WUE	g/liter	377 days	<i>Eucalyptus populnea</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		3.2	0.736	4.72	1.036	0.0	biomass	g	632	174	16	870	239	16	Water use	liters	196	212	586	7	182	310	206
Kelly 2014	Australia	GH	ITE	umol/mmol	301 days	<i>Eucalyptus pilularis</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		4.1	1.71	9.34	2.18	12.0	Photosynthesis	umol/m <sup>2</sup> /s	15.0	2.74	12	25.2	2.82	12	Transpiration	mmol/m <sup>2</sup> /s	4.29	2.14	12	2.85	0.78	12	
Kelly 2014	Australia	GH	ITE	umol/mmol	377 days	<i>Eucalyptus populnea</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		4.0	1.32	12.8	2.17	12.0	Photosynthesis	umol/m <sup>2</sup> /s	16.1	3.31	12	26.6	3.24	12	Transpiration	mmol/m <sup>2</sup> /s	4.33	1.21	12	3.48	1.02	12	
Kelly 2014	Australia	GH	WUE	umol/mol	301 days	<i>Eucalyptus pilularis</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		46.3	12.112	111.4	30.1	12.0	Photosynthesis	umol/m <sup>2</sup> /s	15.0	2.74	12	25.2	2.82	12	St cond	mol/m <sup>2</sup> /s	0.35	0.13	12	0.25	0.08	12	
Kelly 2014	Australia	GH	WUE	umol/mol	377 days	<i>Eucalyptus populnea</i>	C3	Woody	Angio	380	700	ppm	84	H <sub>2</sub> O		45.7	17.812	105.3	31.0	12.0	Photosynthesis	umol/m <sup>2</sup> /s	16.1	3.31	12	26.6	3.24	12	St cond	mol/m <sup>2</sup> /s	0.39	0.14	12	0.27	0.08	12	

Table B2: A list of experiments giving Effect sizes and their respective variances for ambient and elevated CO<sub>2</sub> treatments for iWUE, iTE and WUE. For abbreviations refer to abbreviation list at the end of the table.

References	Location	Facility	Parameter X	Units X	Duration	Plant age	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment	y <sub>ix</sub>	Variance X	Parameter Y	Units Y	y <sub>iy</sub>	Variance Y	Parameter Z	Units Z	y <sub>iz</sub>	Variance Z
Adam, et al., 2000	KN, USA	OTC	iTE	umol/mmol	98 days	178 days	<i>Andropogon gerardii</i>	C4	Herbaceous	Angio	A	2x A	ppm	100	H <sub>2</sub> O	0.60476	0.001628	Photosynthesis	mmol/m <sup>2</sup> /d	0.032281	0.005179	Transpiration	mol/m <sup>2</sup> /d	-0.48395	0.003017
Adam, et al., 2000	KN, USA	OTC	iTE	umol/mmol	98 days	178 days	<i>Andropogon gerardii</i>	C4	Herbaceous	Angio	A	2x A	ppm	100	H <sub>2</sub> O	0.861685	0.00249	Photosynthesis	umol/m <sup>2</sup> /s	0.110003	0.006257	Transpiration	mmol/m <sup>2</sup> /s	-0.56782	0.005529
Albert et al., 2011	Denmark	FACE	iTE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	4 months	Mature	<i>Calluna vulgaris/ Deschampsia flexuosa</i>	C4	Herbaceous	Angio	A	510	ppm	34		0.151834	0.000865	Net Photo	umol/m <sup>2</sup> /s	0.323012	0.000277				
Albert et al., 2011	Denmark	FACE	iTE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	4 months	Mature	<i>Calluna vulgaris/ Deschampsia flexuosa</i>	C4	Herbaceous	Angio	A	510	ppm	34	Temperature	0.253784	0.000412	Net Photo	umol/m <sup>2</sup> /s	0.201015	0.000897				
Hao et al., 2013	China	FACE	iTE	umol/mol	84 days		<i>Isatis indigotica</i>	C3	Herbaceous	Angio	411	550	umol/m <sup>2</sup> /ol	34		0.135007	0.001373	Photosynthesis	umol/m <sup>2</sup> /s	0.144319	0.000741	Transpiration	mmol/m <sup>2</sup> /s	-0.01362	0.001099
Beerling, 1997	Norway	GH	WUE	umol/mol	3 years		<i>Pinus sylvestris</i>	C3	Woody	Gymno	Ambient	560	ppm	47		0.006737									
Fernandez et al., 2002	Venezuela	OTC	iWUE	mol/mol	8 months		<i>Manihot esculenta</i>	C3	Woody	Angio	480	680	umol/m <sup>2</sup> /ol	42		1.217131	0.003718	Photosynthesis	umol/m <sup>2</sup> /s	0.747094	0.001356	St cond	mmol/m <sup>2</sup> /s	-0.63623	0.008758
Ge et al., 2012	Finland	GC	iTE	umol/mmol	135 days		<i>Phalaris arundinacea</i>	C3	Herbaceous	Angio	A	700	umol/m <sup>2</sup> /ol	84	Temperature	0.369885	0.004362	Photosynthesis	umol/m <sup>2</sup> /s	0.286726	0.010196				
Ge et al., 2012	Finland	GC	iTE	umol/mmol	135 days		<i>Phalaris arundinacea</i>	C3	Herbaceous	Angio	A	700	umol/m <sup>2</sup> /ol	84	Temperature	0.422003	0.00966	Photosynthesis	umol/m <sup>2</sup> /s	0.2226	0.012139				
Hileman et al., 1994	AZ, USA	FACE	iTE	mmol/mol	2 growing seasons		<i>Gossypium hirsutum</i>	C3	Crop	Angio	370	550	umol/m <sup>2</sup> /ol	49	H <sub>2</sub> O	0.295932	0.001244	Canopy photo	mmol/m <sup>2</sup> /d	0.288275	0.000868	Canopy transpiration	mol/m <sup>2</sup> /s	-0.01138	0.000514
Ibrahim et al., 2010	Malaysia	GC	iTE	umol/mmol	15 weeks	5 months	<i>Elaeis guineensis</i>	C3	Woody	Angio	400	800	umol/m <sup>2</sup> /ol	100		0.546337	0.01654								
Ibrahim et al., 2010	Malaysia	GC	iTE	umol/mmol	15 weeks	5 months	<i>Elaeis guineensis</i>	C3	Woody	Angio	400	1200	umol/m <sup>2</sup> /ol	200		0.452728	0.021165								
Kellomaki and Wang, 2001	Finland	OTC	iTE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	150 days	4 weeks	<i>Betula Pendula</i>	C3	Woody	Angio	360	700	umol/m <sup>2</sup> /ol	94	Temperature	0.127136	0.004868	Photosynthesis		0.120989	0.005739	Transpiration	mmol/m <sup>2</sup> /s	-0.10992	0.000263
Kellomaki and Wang, 2001	Finland	OTC	iTE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	150 days	4 weeks	<i>Betula Pendula</i>	C3	Woody	Angio	360	700	umol/m <sup>2</sup> /ol	94	Temperature	0.178607	0.004124	Photosynthesis		0.107456	0.005017	Transpiration	mmol/m <sup>2</sup> /s		
Leverenz et al., 1999	Denmark	CTC	iTE	umol/mmol	334 days		<i>Fagus sylvatica</i>	C3	Woody	Angio	40	74	Pa	85	Temperature	0.715003	0.00152	Photosynthesis	umol/m <sup>2</sup> /s	0.30941	0.002339				
Leverenz et al., 1999	Denmark	CTC	iTE	umol/mmol	334 days		<i>Fagus sylvatica</i>	C3	Woody	Angio	40	74	Pa	85	Temperature	0.750605	0.001687	Photosynthesis	umol/m <sup>2</sup> /s	0.428377	0.002917				
Leverenz et al., 1999	Denmark	CTC	iTE	umol/mmol	334 days		<i>Fagus sylvatica</i>	C3	Woody	Angio	40	74	Pa	85	Temperature	0.940339	0.002112	Photosynthesis	umol/m <sup>2</sup> /s	0.536141	0.002125				
Leverenz et al., 1999	Denmark	CTC	iTE	umol/mmol	334 days		<i>Fagus sylvatica</i>	C3	Woody	Angio	40	74	Pa	85	Temperature	0.827389	0.002308	Photosynthesis	umol/m <sup>2</sup> /s	0.640199	0.003046				
Lewis et al., 2002	OR, USA	GC	iTE	mmol CO <sub>2</sub> / mol H <sub>2</sub> O	21 months	2 years	<i>Pseudotsuga menziesii</i>	C3	Woody	Gymno	A	A+200	umol/m <sup>2</sup> /ol	53		0.310408	0.001262	Photosynthesis	umol/m <sup>2</sup> /s	0.178190	0.000658	Transpiration	mmol/m <sup>2</sup> /s	-0.10856	0.001297
Liang and Maruyama, 1995	Japan	GC	iTE	mmol/mol	4 months	seeds	<i>Alnus firma</i>	C3	Woody	Angio	350	900	umol/m <sup>2</sup> /ol	100	H <sub>2</sub> O	0.328180	0.008293	Photosynthesis	umol/m <sup>2</sup> /s	0.496778	0.012414	Transpiration	mmol/m <sup>2</sup> /s	-0.08192	0.002687
Liang and Maruyama, 1995	Japan	GC	iTE	mmol/mol	4 months	seeds	<i>Alnus firma</i>	C3	Woody	Angio	350	900	umol/m <sup>2</sup> /ol	157	H <sub>2</sub> O	0.418709	0.01029	Photosynthesis	umol/m <sup>2</sup> /s	0.689268	0.011622	Transpiration	mmol/m <sup>2</sup> /s	-0.21667	0.002857
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Sequoia sempervirens</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.276930	0.008711					Transpiration	mol of H <sub>2</sub> O/plant/d	-0.12563	0.003651
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Metasequoia glyptostroboides</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.194136	0.016642					Transpiration	mol of H <sub>2</sub> O/plant/d	-0.07646	0.008933
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Taxodium distichum</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.487882	0.010039					Transpiration	mol of H <sub>2</sub> O/plant/d	-0.19516	0.004043
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Sequoia sempervirens</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.571146	0.00454								
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Metasequoia glyptostroboides</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.595004	0.010559								
Llorens et al., 2009	UK	GC	iTE	mmol/mol	3 years	1 month	<i>Taxodium distichum</i>	C3	Woody	Gymno	400	800	umol/m <sup>2</sup> /ol	100		0.535508	0.005672								
Maroco eta la., 1999	WA, USA	GC	iWUE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	30 days	seeds	<i>Zea mays</i>	C4	Crop	Angio	350	1100	uW	214		1.06695	0.004063					St cond	mmol/m <sup>2</sup> /s	-1.1785	0.032546

References	Location	Facility	Parameter X	Units X	Duration	Plant age	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment	yix	Variance X	Parameter Y	Units Y	yiy	Variance Y	Parameter Z	Units Z	yiz	Variance Z
Rey and Jarvis, 1998	UK	OTC	ITE	mmol/mol	3 growing seasons	1 year	<i>Betula pendula</i>	C3	Woody	Angio	350	700	umol/m <sup>2</sup> /ol	100		-0.43415	0.008511					Transpiration	mmol/m <sup>2</sup> /s	-0.21383	0.009834
Tausz-Pösch et al., 2013	Australia	FACE	ITE	mmol/mol	4 months		<i>Triticum aestivum</i>	C4	Crop	Angio	390	550	umol/m <sup>2</sup> /ol	41		0.412305	0.001044	Photosynthesis	mol/m <sup>2</sup> /day	0.152373	0.002181	Transpiration	mol/m <sup>2</sup> /day	-0.2654	0.003412
Tausz-Pösch et al., 2013	Australia	FACE	ITE	mmol/mol	4 months		<i>Triticum aestivum</i>	C4	Crop	Angio	390	550	umol/m <sup>2</sup> /ol	41		0.487993	0.000641	Photosynthesis	mol/m <sup>2</sup> /day	0.191346	0.001791	Transpiration	mol/m <sup>2</sup> /day	-0.26586	0.002211
Tausz-Pösch et al., 2013	Australia	FACE	IWUE	umol/mol	4 months		<i>Triticum aestivum</i>	C4	Crop	Angio	390	550	umol/m <sup>2</sup> /ol	41		0.327382	0.00212	Photosynthesis	umol/m <sup>2</sup> /s	0.152565	0.004831	St cond	mol/m <sup>2</sup> /s	-0.23406	0.003276
Tausz-Pösch et al., 2013	Australia	FACE	IWUE	umol/mol	4 months		<i>Triticum aestivum</i>	C4	Crop	Angio	390	550	umol/m <sup>2</sup> /ol	41		0.354097	0.00235	Photosynthesis	umol/m <sup>2</sup> /s	0.107989	0.004207	St cond	mol/m <sup>2</sup> /s	-0.26449	0.002973
Centritto et al., 1999a	UK	OTC	ITE	mmol/mol	2 growing seasons	0 days	<i>Prunus avium</i>	C3	Woody	Angio	350	700	umol/m <sup>2</sup> /ol	100	H <sub>2</sub> O	0.731984	0.00067	Net Photo	umol/m <sup>2</sup> /s	0.592301	0.000662				
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	seeds	<i>Agrostemma githago</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.36569	0.02227								
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	seeds	<i>Chenopodium album</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.252283	0.023834								
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	seeds	<i>Sinapis arvensis</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.066167	0.129368								
Erbs et al., 2009	Germany	FACE	ITE	mmol/mol	3 years	seeds	<i>Triticum aestivum</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.261488	0.046419								
Erbs et al., 2009	Germany	FACE	IWUE	mmol/mol	3 years	seeds	<i>Agrostemma githago</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.444306	0.014697								
Erbs et al., 2009	Germany	FACE	IWUE	mmol/mol	3 years	seeds	<i>Chenopodium album</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.384147	0.042813								
Erbs et al., 2009	Germany	FACE	IWUE	mmol/mol	3 years	seeds	<i>Triticum aestivum</i>	C4	Herbaceous	Angio	A	A+150	umol/m <sup>2</sup> /ol	39		0.249492	0.047976								
Fredeen and Field, 1995	CA, USA	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	50 days	2 years	<i>Avena sativa</i>	C4	Herbaceous	Angio	A	A+35	Pa	92		0.440993	0.01823	Photosynthesis	umol/m <sup>2</sup> /s	0.550195	0.011898	Transpiration	mmol/m <sup>2</sup> /s	-0.2535	0.022276
Fredeen and Field, 1995	CA, USA	GC	ITE	umol CO <sub>2</sub> /mmol H <sub>2</sub> O	50 days	2 years	<i>Avena sativa</i>	C4	Herbaceous	Angio	A	A+35	Pa	92		1.10447	0.00632	Photosynthesis	umol/m <sup>2</sup> /s	0.7147	0.005537	EvapoTrans	mmol/m <sup>2</sup> /s	-0.20854	0.000469
Norby and O'Neill, 1991	TN, USA	GH	ITE	mmol/mol	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	493	cm <sup>3</sup> /m <sup>3</sup>	33	Nutrient	0.174834	0.008912	Photosynthesis	umol/m <sup>2</sup> /s	0.2641	0.007142				
Norby and O'Neill, 1991	TN, USA	GH	ITE	mmol/mol	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	493	cm <sup>3</sup> /m <sup>3</sup>	33	Nutrient	-0.24447	0.006069	Photosynthesis	umol/m <sup>2</sup> /s	0.169301	0.002564				
Norby and O'Neill, 1991	TN, USA	GH	ITE	mmol/mol	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	787	cm <sup>3</sup> /m <sup>3</sup>	112	Nutrient	-0.1934	0.006896	Photosynthesis	umol/m <sup>2</sup> /s	0.504234	0.00941				
Norby and O'Neill, 1991	TN, USA	GH	ITE	mmol/mol	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	787	cm <sup>3</sup> /m <sup>3</sup>	112	Nutrient	-0.08111	0.008973	Photosynthesis	umol/m <sup>2</sup> /s	0.296245	0.003566				
Norby and O'Neill, 1991	TN, USA	GH	WUE	g/cm <sup>3</sup>	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	493	cm <sup>3</sup> /m <sup>3</sup>	33	Nutrient	0.201194	0.000128					Water use	cm <sup>3</sup> /d	-0.03984	0.000988
Norby and O'Neill, 1991	TN, USA	GH	WUE	g/cm <sup>3</sup>	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	493	cm <sup>3</sup> /m <sup>3</sup>	33	Nutrient	0.18462	0.003323					Water use	cm <sup>3</sup> /d	0.026093	0.006906
Norby and O'Neill, 1991	TN, USA	GH	WUE	g/cm <sup>3</sup>	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	787	cm <sup>3</sup> /m <sup>3</sup>	112	Nutrient	0.425340	0.000124					Water use	cm <sup>3</sup> /d	-0.34773	0.003413
Norby and O'Neill, 1991	TN, USA	GH	WUE	g/cm <sup>3</sup>	168 days	1 week	<i>Linodendron tulipifera</i>	C3	Woody	Angio	371	787	cm <sup>3</sup> /m <sup>3</sup>	112	Nutrient	0.185306	0.002484					Water use	cm <sup>3</sup> /d	-0.07862	0.010872
Barton et al, 2011	Australia	WTC	ITE	umol/mmol	2 years	5 years	<i>Eucalyptus saligna</i>	C3	Woody	Angio	380	620	ppm	63		0.454596	0.000904	Photosynthesis	umol/m <sup>2</sup> /s	0.128295	0.000962	Transpiration	mmol/m <sup>2</sup> /s	-0.27494	0.00148
Dixon et al., 1995	France	OTC	IWUE	umol/mol	180 days	8 years old	<i>Picea abies</i>	C3	Woody	Gymno	350	740	umol/m <sup>2</sup> /ol	111		0.138104	0.000418	Photosynthesis	umol/m <sup>2</sup> /s	0.383576	0.003179	St cond	mol/m <sup>2</sup> /s	-0.06803	0.004439
Dixon et al., 1995	France	OTC	IWUE	umol/mol	180 days	5 years old	<i>Quercus rubra</i>	C3	Woody	Angio	350	740	umol/m <sup>2</sup> /ol	111		0.144708	0.001037	Photosynthesis	umol/m <sup>2</sup> /s	0.428294	0.004606	St cond	mol/m <sup>2</sup> /s	0.288427	0.004082
Picon et al., 1997	France	GH	IWUE	mmol/mol	222 days		<i>Quercus robur</i>	C3	Woody	Angio	350	700	umol/m <sup>2</sup> /ol	100	H <sub>2</sub> O	0.667338	0.0007	Photosynthesis	umol/m <sup>2</sup> /s	0.423382	0.000919	St cond	mmol/m <sup>2</sup> /s	-0.20879	0.001511
Tissue et al., 1997	NC, USA	OTC	IWUE	mol/mol	4 years	1 month	<i>Pinus taeda</i>	C3	Woody	Gymno	35	65	Pa	86		0.75275	0.00032	Photosynthesis	umol/m <sup>2</sup> /s	0.501078	0.000277	St cond	mmol/m <sup>2</sup> /s	-0.25692	0.000736
Drake, 1992	MD, USA	OTC	WUE	mol/mol	4 years		<i>Scirpus olneyi</i>	C3	Herbaceous	Angio	340	681	ppm	100		0.559841	0.001494	Net carbon exchange	kg/m <sup>2</sup> /year	0.410500	0.000866	EvapoTrans		-0.20162	0.000434
Drake, 1992	MD, USA	OTC	WUE	mol/mol	4 years		<i>Spartina patens</i>	C4	Herbaceous	Angio	340	681	ppm	100		0.58970	0.004829	Net carbon	kg/m <sup>2</sup> /year	0.187980	0.000242	EvapoTrans		-	0.000776

References	Location	Facility	Parameter X	Units X	Duration	Plant age	Species	C3 or C4	Functional Division 1	Functional Division 2	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	CO <sub>2</sub> Unit	% Increment	Interacting treatment	y <sub>ix</sub>	Variance X	Parameter Y	Units Y	y <sub>iy</sub>	Variance Y	Parameter Z	Units Z	y <sub>iz</sub>	Variance Z	
																8		exchange	μmol/m <sup>2</sup> /s	0.29646	0.000236			0.32864		
Tognetti et al., 1999	Italy	FACE	ITE	μmol/mmol	1 growing season		<i>Populus euramericana</i>	C3	Woody	Angio	360	560	μmol/mol	56			0.39153	0.000447	Photosynthesis	μmol/m <sup>2</sup> /s	0.000236	Transpiration	mmol/m <sup>2</sup> /s	-0.03124	5.8E-05	
Tognetti et al., 1999	Italy	FACE	ITE	μmol/mmol	1 growing season		<i>Populus deltoides</i>	C3	Woody	Angio	360	560	μmol/mol	56			0.43423	0.001028	Photosynthesis	μmol/m <sup>2</sup> /s	0.35694	0.000156	Transpiration	mmol/m <sup>2</sup> /s	-0.08317	0.00026
Conley et al., 2001	AZ, USA	FACE	WUE	g/m <sup>2</sup> /mm	2 years	culture	<i>Sorghum bicolor</i>	C4	Crop	Angio	370	570	μmol/mol	54	H <sub>2</sub> O		0.09329	0.004859	Biomass	g/m <sup>2</sup>	0.03953	0.000451	Evapotrans	mm	-0.09447	0.00191
Elsworth 1999	NC, USA	FACE	ITE	mmol/mol	2 years	16 years	<i>Pinus taeda</i>	C3	Woody	Gymno	Ambient	A+200	μmol/mol	53			0.57584	0.000336	Photosynthesis	μmol/m <sup>2</sup> /s	0.49247	0.003059				
Udding et. Al., 2009	Rhineland	FACE	WUE	μmol/mol			<i>Betula papyrifera</i>	C3	Woody	Angio	Ambient	560	ppm	45			0.004889	0.004602	Photosynthesis	μmol/m <sup>2</sup> /s	0.13705	0.004602	St cond	mol/m <sup>2</sup> /s	0.16642	0.00923
Udding et. Al., 2009	Rhineland	FACE	WUE	μmol/mol			<i>Populus tremuloides</i>	C3	Woody	Angio	Ambient	561	ppm	45			0.005054	0.003963	Photosynthesis	μmol/m <sup>2</sup> /s	0.08131	0.003963	St cond	mol/m <sup>2</sup> /s	0.18254	0.007662
Barton et al., 2011	Glencorse	OTC	WUE	μmol/mol			<i>Picea sitchensis</i>	C3	Woody	Gymno	Ambient	700	μmol/mol	84			0.003719	0.006178	Photosynthesis	μmol/m <sup>2</sup> /s	0.20267	0.006178	St cond	mol/m <sup>2</sup> /s	-0.03676	0.003163
Broadmeadow M.	Healdley	OTC	WUE	μmol/mol			<i>Quercus petraea</i>	C3	Woody	Angio	350	700	μmol/mol	100			0.45013	0.00089	Photosynthesis	μmol/m <sup>2</sup> /s	0.06354	0.001066	St cond	mol/m <sup>2</sup> /s	-0.29777	0.000843
Rey and Jarvis, 1997	Glencorse	OTC	ITE	mmol/mol			<i>Betula pendula</i>	C3	Woody	Angio	350	700	μmol/mol	100			0.41363	0.004173	Photosynthesis	μmol/m <sup>2</sup> /s	0.2691	0.001745	Transpiration	mmol/m <sup>2</sup> /s	-0.19724	0.004017
Rey and Jarvis, 1997	Glencorse	OTC	WUE	μmol/mol			<i>Betula pendula</i>	C3	Woody	Angio	350	700	μmol/mol	100			0.57188	0.004169	Photosynthesis	μmol/m <sup>2</sup> /s	0.2691	0.001745	St cond	mol/m <sup>2</sup> /s	-0.36536	0.006193
Warren et al., 2011	TN, USA	FACE	WUE	g/m <sup>2</sup> /y/mm	4 growing seasons	1 year old	<i>Liquidambar styraciflua</i>	C3	Woody	Angio	Ambient	A+200	μmol/mol	53			0.32089	0.001357	NPP	g DM/m <sup>2</sup> /y	0.127683	0.001399	Transpiration	mm	-0.1862	0.000142
Elsworth D.	NC, USA	FACE	WUE	g/m <sup>2</sup> /y/mm	10 years	16 years	<i>Pinus taeda</i>	C3	Woody	Gymno	Ambient	A+200	μmol/mol	53			0.25228	0.000825	NPP	g DM/m <sup>2</sup> /y	0.263858	0.00044	Transpiration	mm	0.01471	0.001797

## Abbreviations used in Table B1 & B2:

Code	Abbreviation	Code	Abbreviation
GC	Growth chamber	XSdE	Elevated Standard deviation iWUE, iTE, WUE
TGG	Temperature gradient greenhouse	XNE	Elevated Replicates iWUE, iTE, WUE
GH	Green house	YA	Ambient Mean Photosynthesis, Biomass
OTC	Open top Chamber	YSdA	Ambient Standard deviation Photosynthesis, Biomass
OTM	Open top mesocosm	YNA	Ambient Replicates Photosynthesis, Biomass
GT	Growth tunnel	YE	Elevated Mean Photosynthesis, Biomass
FACE	Free air Carbon dioxide enrichment	YSdE	Elevated Standard deviation Photosynthesis, Biomass
CTC	Close top chamber	YNE	Elevated Replicates Photosynthesis, Biomass
WTC	Whole tree chamber	ZA	Ambient Mean Stomatal Conductance, Transpiration
LYCOG	Ly simeter CO <sub>2</sub> gradient	ZSdA	Ambient Standard deviation Stomatal Conductance/ Transpiration
BB	Branch Bag	ZNA	Ambient Replicates Stomatal Conductance, Transpiration
WUE	Water Use efficiency	ZE	Elevated Mean Stomatal Conductance, Transpiration
iWUE	intrinsic water use efficiency	ZSdE	Elevated Standard deviation Stomatal Conductance, Transpiration
iTE	instantaneous transpiration efficiency	ZNE	Elevated Replicates Stomatal Conductance, Transpiration
XA	Ambient Mean iWUE, iTE, WUE	y <sub>ix</sub>	Mean Effect size iWUE, iTE, WUE
XSdA	Ambient Standard deviation iWUE, iTE, WUE	y <sub>iy</sub>	Mean Effect size Photosynthesis, Biomass
XNA	Ambient Replicates iWUE, iTE, WUE	y <sub>iz</sub>	Mean Effect size Stomatal conductance, Transpiration
XE	Elevated Mean iWUE, iTE, WUE		

## References (Table B1):

References	
Anderson et al., 1998	Anderson, P. D. and P. T. Tomlinson. 1998. Ontogeny affects response of northern red oak seedlings to elevated CO <sub>2</sub> and water stress - I. Carbon assimilation and biomass production. <i>New Phytologist</i> 140:477-491.
Aranjuelo et al., 2006	Aranjuelo, I., J. J. Irigoyen, P. Perez, R. Martinez-Carrasco, and M. Sanchez-Diaz. 2006. Response of nodulated alfalfa to water supply, temperature and elevated CO <sub>2</sub> : productivity and water relations. <i>Environmental and Experimental Botany</i> 55:130-141.
Atkinson et al., 1997	Atkinson, C. J., J. M. Taylor, D. Wilkins, and R. T. Besford. 1997. Effects of elevated CO <sub>2</sub> on chloroplast components, gas exchange and growth of oak and cherry. <i>Tree Physiology</i> 17:319-325.
Ball et al., 1997	Ball, M. C., M. J. Cochrane, and H. M. Rawson. 1997. Growth and water use of the mangroves <i>Rhizophora apiculata</i> and <i>R-stylosa</i> in response to salinity and humidity under ambient and elevated concentrations of atmospheric CO <sub>2</sub> . <i>Plant Cell and Environment</i> 20:1158-1166.
Biswas et al., 2013	Biswas, D. K., H. Xu, Y. G. Li, B. L. Ma, and G. M. Jiang. 2013. Modification of photosynthesis and growth responses to elevated CO <sub>2</sub> by ozone in two cultivars of winter wheat with different years of release. <i>Journal of Experimental Botany</i> 64:1485-1496.
Bucher-Wallin et al., 2000	Bucher-Wallin, I. K., M. A. Sonnleithner, P. Egli, M. S. Gunthardt-Goerg, D. Tarjan, R. Schulin, and J. B. Bucher. 2000. Effects of elevated CO <sub>2</sub> , increased nitrogen deposition and soil on evapotranspiration and water use efficiency of spruce-beech model ecosystems. <i>Phyton-Annales Rei Botanicae</i> 40:49-60.
Bunce, 2003	Bunce, J. A. 2003. Effects of water vapor pressure difference on leaf gas exchange in potato and sorghum at ambient and elevated carbon dioxide under field conditions. <i>Field Crops Research</i> 82:37-47.
Cao et al., 2007	Cao, B., Q. L. Dang, and S. R. Zhang. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO <sub>2</sub> ] in white birch seedlings. <i>Tree Physiology</i> 27:891-899.
Carter et al., 1997	Carter, E. B., M. K. Theodorou, and P. Morris. 1997. Responses of <i>Lotus corniculatus</i> to environmental change. 1. Effects of elevated CO <sub>2</sub> , temperature and drought on growth and plant development. <i>New Phytologist</i> 136:245-253.
Causin et al., 2006	Causin, H. F., T. W. Ruffy, and J. F. Reynolds. 2006. Gas exchange and carbon metabolism in two <i>Prosopis</i> species (Fabaceae) from semiarid habitats: Effects of elevated CO <sub>2</sub> , N supply, and N source. <i>American Journal of Botany</i> 93:716-723.
Centritto et al., 1999a	Centritto, M., H. S. J. Lee, and P. G. Jarvis. 1999a. Interactive effects of elevated [CO <sub>2</sub> ] and drought on cherry ( <i>Prunus avium</i> ) seedlings - I. Growth, whole-plant water use efficiency and water loss. <i>New Phytologist</i> 141:129-140.
Centritto et al., 2002	Centritto, M., M. E. Lucas, and P. G. Jarvis. 2002. Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach ( <i>Prunus persica</i> ) seedlings in response to elevated carbon dioxide concentration and water availability. <i>Tree Physiology</i> 22:699-706.
Cernusak et al., 2011	Cernusak, L. A., K. Winter, C. Martinez, E. Correa, J. Aranda, M. Garcia, C. Jaramillo, and B. L. Turner. 2011. Responses of Legume Versus Nonlegume Tropical Tree Seedlings to Elevated CO <sub>2</sub> Concentration. <i>Plant Physiology</i> 157:372-385.
Ceusters et al 2008	Ceusters, J., A. M. Borland, E. Londers, V. Verdoodt, C. Godts, and M. P. De Proft. 2008. Diel shifts in carboxylation pathway and metabolite dynamics in the CAM bromeliad <i>Aechmea 'Maya'</i> in response to elevated CO <sub>2</sub> . <i>Annals of Botany</i> 102:389-397.
Chen et al., 1999	Chen, K., G. Q. Ha, N. Keutgen, M. J. J. Janssens, and F. Lenz. 1999. Effects of NaCl salinity and CO <sub>2</sub> enrichment on pepino ( <i>Solanum muricatum</i> Ait.) - I. Growth and yield. <i>Scientia Horticulturae</i> 81:25-41.
Choi et al., 2005	Choi, D. S., A. M. Qureshi, Y. Maruyama, H. O. Jin, and T. Koike. 2005. Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of <i>Pinus densiflora</i> seedlings grown under elevated CO <sub>2</sub> concentrations. <i>Photosynthetica</i> 43:223-229.
Clifford et al., 2000	Clifford, S. C., I. M. Stronach, C. R. Black, P. R. Singleton-Jones, S. N. Azam-Ali, and N. M. J. Crout. 2000. Effects of elevated CO <sub>2</sub> , drought and temperature on the water relations and gas exchange of groundnut ( <i>Arachis hypogaea</i> ) stands grown in controlled environment glasshouses. <i>Physiologia Plantarum</i> 110:78-88.
Coleman and Bazzaz, 1992	Coleman, J. S. and F. A. Bazzaz. 1992. Effects of CO <sub>2</sub> and Temperature on Growth and Resource Use of Cooccurring C3 and C4 Annuals. <i>Ecology</i> 73:1244-1259.
Crous et al., 2010	Requested
Dany agri and Dand, 2013	Dany agri, G. and Q. L. Dang. 2013. Effects of Elevated [CO <sub>2</sub> ] and Low Soil Moisture on the Physiological Responses of Mountain Maple ( <i>Acer spicatum</i> L.) Seedlings to Light. <i>Plos One</i> 8.
deAngelis	Requested
DeLuis et al., 1999	De Luis, I., J. J. Irigoyen, and M. Sanchez-Diaz. 1999. Elevated CO <sub>2</sub> enhances plant growth in droughted N-2-fixing alfalfa without improving water status. <i>Physiologia Plantarum</i> 107:84-89.
Diemer, 1994	Diemer, M. W. 1994. Mid-Season Gas-Exchange of an Alpine Grassland under Elevated CO <sub>2</sub> . <i>Oecologia</i> 98:429-435.
Ellsworth 1999	Ellsworth, D. S. 1999. CO <sub>2</sub> enrichment in a maturing pine forest: are CO <sub>2</sub> exchange and water status in the canopy affected? <i>Plant Cell and Environment</i> 22:461-472.
Ellsworth	Requested
Erbs et al., 2009	Erbs, M., J. Franzaring, P. Hogg, and A. Fangmeier. 2009. Free-air CO <sub>2</sub> enrichment in a wheat-weed assembly - effects on water relations. <i>Basic and Applied Ecology</i> 10:358-367.
Fay et al., 2009	Fay, P. A., A. M. Kelley, A. C. Procter, D. F. Hui, V. L. Jin, R. B. Jackson, H. B. Johnson, and H. W. Polley. 2009. Primary Productivity and Water Balance of Grassland Vegetation on Three Soils in a Continuous CO <sub>2</sub> Gradient: Initial Results from the Ly simeter CO <sub>2</sub> Gradient Experiment. <i>Ecosystems</i> 12:699-714.
Ferris and Taylor, 1995	Ferris, R. and G. Taylor. 1995. Contrasting effects of elevated CO <sub>2</sub> and water deficit on two native herbs. <i>New Phytologist</i> 131:491-501.
Franzaring et al., 2011	Franzaring, J., S. Weller, I. Schmid, and A. Fangmeier. 2011. Growth, senescence and water use efficiency of spring oilseed rape ( <i>Brassica napus</i> L. cv. Mozart) grown in a factorial combination of nitrogen supply and elevated CO <sub>2</sub> . <i>Environmental and Experimental Botany</i> 72:284-296.
Fredeen et al., 1998	Fredeen, A. L., G. W. Koch, and C. B. Field. 1998. Influence of fertilization and atmospheric CO <sub>2</sub> enrichment on ecosystem CO <sub>2</sub> and H <sub>2</sub> O exchanges in single- and multiple-species grassland microcosms. <i>Environmental and Experimental Botany</i> 40:147-157.
Freeman 1998	Freeman, M. 1998. Leaf Gas Exchange in Mature Beech ( <i>Fagus sylvatica</i> L.) Exposed to Long-term Elevated CO <sub>2</sub> in Branch Bags: Royal Veterinary and Agricultural University, Department of Botany, Dendrology and Forest Genetics, Arboretum.
Geissler et al., 2009	Geissler, N., S. Hussin, and H. W. Koyro. 2009. Elevated atmospheric CO <sub>2</sub> concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of <i>Aster tripolium</i> L. <i>Journal of Experimental Botany</i> 60:137-151.

References	
Ghannoum et al., 2001	Ghannoum, O., S. von Caemmerer, and J. P. Conroy. 2001. Plant water use efficiency of 17 Australian NAD-ME and NADP-ME C-4 grasses at ambient and elevated CO <sub>2</sub> partial pressure. <i>Australian Journal of Plant Physiology</i> 28:1207-1217.
Ghannoum et al., 2010	Ghannoum, O., N. G. Phillips, M. A. Sears, B. A. Logan, J. D. Lewis, J. P. Conroy, and D. T. Tissue. 2010. Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO <sub>2</sub> ] and temperature. <i>Plant Cell and Environment</i> 33:1671-1681.
Ghasemzadeh and Jaafar, 2011	Ghasemzadeh, A. and H. Z. E. Jaafar. 2011. Effect of CO <sub>2</sub> Enrichment on Synthesis of Some Primary and Secondary Metabolites in Ginger ( <i>Zingiber officinale Roscoe</i> ). <i>International Journal of Molecular Sciences</i> 12:1101-1114.
Gorissen et al., 1995	Gorissen, A., P. J. Kuikman, and H. Vandebeek. 1995. Carbon Allocation and Water-Use in Juvenile Douglas-Fir under Elevated CO <sub>2</sub> . <i>New Phytologist</i> 129:275-282.
Greenep et al., 2003	Greenep, H., M. H. Turnbull, and D. Whitehead. 2003. Response of photosynthesis in second-generation <i>Pinus radiata</i> trees to long-term exposure to elevated carbon dioxide partial pressure. <i>Tree Physiology</i> 23:569-576.
Grunzweig et al., 2003	Grunzweig, J. M. and C. Körner. 2003. Differential phosphorus and nitrogen effects drive species and community responses to elevated CO <sub>2</sub> in semi-arid grassland. <i>Functional Ecology</i> 17:766-777.
Guehl et al., 1994	Guehl, J. M., C. Picon, G. Aussenac, and P. Gross. 1994. Interactive Effects of Elevated CO <sub>2</sub> and Soil Drought on Growth and Transpiration Efficiency and Its Determinants in 2 European Forest Tree Species. <i>Tree Physiology</i> 14:707-724.
Gunderson et al., 2002	Gunderson, C. A., J. D. Sholtis, S. D. Wulschleger, D. T. Tissue, P. J. Hanson, and R. J. Norby. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum ( <i>Liquidambar styraciflua</i> L.) plantation during 3 years of CO <sub>2</sub> enrichment. <i>Plant Cell and Environment</i> 25:379-393.
Haworth et al., 2011	Haworth, M., C. Elliott-Kingston, and J. C. McElwain. 2011. The stomatal CO <sub>2</sub> proxy does not saturate at high atmospheric CO <sub>2</sub> concentrations: evidence from stomatal index responses of Araucariaceae conifers. <i>Oecologia</i> 167:11-19.
Hirano et al., 2012	Hirano, A., I. Hongo, and T. Koike. 2012. Morphological and physiological responses of Siebold's beech ( <i>Fagus crenata</i> ) seedlings grown under CO <sub>2</sub> concentrations ranging from pre-industrial to expected future levels. <i>Landscape and Ecological Engineering</i> 8:59-67.
Igamberdiev et al., 2004	Igamberdiev, A. U., T. N. Mikkelsen, P. Ambus, H. Bauwe, P. J. Lea, and P. Gardestrom. 2004. Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato and Arabidopsis plants deficient in glycine decarboxylase. <i>Photosynthesis Research</i> 81:139-152.
Jensen and Christensen, 2004	Jensen, B. and B. T. Christensen. 2004. Interactions between elevated CO <sub>2</sub> and added N: Effects on water use, biomass, and soil N-15 uptake in wheat. <i>Acta Agriculturae Scandinavica Section B-Soil and Plant Science</i> 54:175-184.
Kelly J. W.	Kelly J. W. 2014. Thesis: Productivity and Water use of Australian tree species under Climate Change. Submitted to Macquarie University, Sydney, Australia.
Keutgen and Chen, 2001	Keutgen, N. and K. Chen. 2001. Responses of citrus leaf photosynthesis, chlorophyll fluorescence, macronutrient and carbohydrate contents to elevated CO <sub>2</sub> . <i>Journal of Plant Physiology</i> 158:1307-1316.
Khurana and Singh, 2004	Khurana, E. and J. S. Singh. 2004. Response of five dry tropical tree seedlings to elevated CO <sub>2</sub> : Impact of seed size and successional status. <i>New Forests</i> 27:139-157.
Kim et al., 2006	Kim, S. H., R. C. Sicher, H. Bae, D. C. Gitz, J. T. Baker, D. J. Timlin, and V. R. Reddy. 2006. Canopy photosynthesis, evapotranspiration, leaf nitrogen, and transcription profiles of maize in response to CO <sub>2</sub> enrichment. <i>Global Change Biology</i> 12:588-600.
Klus et al., 2001	Klus, D. J., S. Kalisz, P. S. Curtis, J. A. Teeri, and S. J. Tonsor. 2001. Family- and population-level responses to atmospheric CO <sub>2</sub> concentration: Gas exchange and the allocation of C, N, and biomass in <i>Plantago lanceolata</i> (Plantaginaceae). <i>American Journal of Botany</i> 88:1080-1087.
Koike et al., 1996	Koike, T., T. T. Lei, T. C. Maximov, R. Tabuchi, K. Takahashi, and B. I. Ivanov. 1996. Comparison of the photosynthetic capacity of Siberian and Japanese birch seedlings grown in elevated CO <sub>2</sub> and temperature. <i>Tree Physiology</i> 16:381-385.
Kubiske and Pregitzer, 1997	Kubiske, M. E. and K. S. Pregitzer. 1997. Ecophysiological responses to simulated canopy gaps of two tree species of contrasting shade tolerance in elevated CO <sub>2</sub> . <i>Functional Ecology</i> 11:24-32.
Leavitt et al., 2003	Leavitt, S. W., S. B. Idso, B. A. Kimball, J. M. Burns, A. Sinha, and L. Stott. 2003. The effect of long-term atmospheric CO <sub>2</sub> enrichment on the intrinsic water-use efficiency of sour orange trees. <i>Chemosphere</i> 50:217-222.
LeThiec and Dixon, 1996	LeThiec, D. and M. Dixon. 1996. Acclimation of photosynthesis in Norway spruce and red oak grown in open-top chambers and subjected to natural drought and to elevated CO <sub>2</sub> . <i>Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere</i> 26:87-94.
Li et al., 2003	Li, F. S., S. Z. Kang, J. H. Zhang, and S. Cohen. 2003. Effects of atmospheric CO <sub>2</sub> enrichment, water status and applied nitrogen on water- and nitrogen-use efficiencies of wheat. <i>Plant and Soil</i> 254:279-289.
Li et al., 2010	Li, J. H., J. E. Erickson, G. Peresta, and B. G. Drake. 2010. Evapotranspiration and water use efficiency in a Chesapeake Bay wetland under carbon dioxide enrichment. <i>Global Change Biology</i> 16:234-245.
Li et al., 2013	Li, D. X., H. L. Liu, Y. Z. Qiao, Y. N. Wang, Z. M. Cai, B. D. Dong, C. H. Shi, Y. Y. Liu, X. Li, and M. Y. Liu. 2013. Effects of elevated CO <sub>2</sub> on the growth, seed yield, and water use efficiency of soybean ( <i>Glycine max</i> (L.) Merr.) under drought stress. <i>Agricultural Water Management</i> 129:105-112.
Lodge et al., 2001	Lodge, R. J., P. Dijkstra, B. G. Drake, and J. I. L. Morison. 2001. Stomatal acclimation to increased CO <sub>2</sub> concentration in a Florida scrub oak species <i>Quercus myrtifolia</i> Willd. <i>Plant Cell and Environment</i> 24:77-88.
Magliulo et al., 2003	Magliulo, V., M. Bindi, and G. Rana. 2003. Water use of irrigated potato ( <i>Solanum tuberosum</i> L.) grown under free air carbon dioxide enrichment in central Italy. <i>Agriculture Ecosystems &amp; Environment</i> 97:65-80.
Manderscheid and Weigel, 2007	Manderscheid, R. and H. J. Weigel. 2007. Drought stress effects on wheat are mitigated by atmospheric CO <sub>2</sub> enrichment. <i>Agronomy for Sustainable Development</i> 27:79-87.
Mark and Tevini., 1996	Mark, U. and M. Tevini. 1997. Effects of solar ultraviolet-B radiation, temperature and CO <sub>2</sub> on growth and physiology of sunflower and maize seedlings. <i>Plant Ecology</i> 128:224-234.
Murthy et al., 2005	Murthy, R., G. Barron-Gafford, P. M. Dougherty, V. C. Engel, K. Grieve, L. Handley, C. Klimas, M. J. Potosnak, S. J. Zarnoch, and J. W. Zhang. 2005. Increased leaf area dominates carbon flux response to elevated CO <sub>2</sub> in stands of <i>Populus deltoides</i> (Bartr.). <i>Global Change Biology</i> 11:716-731.
Norby et al., 1986	Norby, R. J., E. G. Oneill, and R. J. Luxmoore. 1986. Effects of Atmospheric CO <sub>2</sub> Enrichment on the Growth and Mineral-Nutrition of Quercus-Alba Seedlings in Nutrient-Poor Soil. <i>Plant Physiology</i> 82:83-89.
Novriyanti et al., 2012	Novriyanti, E., M. Watanabe, M. Kitao, H. Utsugi, A. Uemura, and T. Koike. 2012. High nitrogen and elevated [CO <sub>2</sub> ] effects on the growth, defense and photosynthetic performance of two eucalypt species. <i>Environmental Pollution</i> 170:124-130.
Perez-Lopez et al., 2009	Perez-Lopez, U., A. Robredo, M. Lacuesta, A. Mena-Petite, and A. Munoz-Rueda. 2009. The impact of salt stress on the water status of barley plants is partially mitigated by elevated CO <sub>2</sub> . <i>Environmental and Experimental Botany</i> 66:463-470.
Pettersson and McDonald, 1992	Pettersson, R. and A. J. S. McDonald. 1992. Effects of Elevated Carbon-Dioxide Concentration on Photosynthesis and Growth of Small Birch Plants ( <i>Betula-Pendula Roth</i> ) at Optimal Nutrition. <i>Plant Cell and Environment</i> 15:911-919.
Picon et al., 1996	Picon, C., J. M. Guehl, and G. Aussenac. 1996. Growth dynamics, transpiration and water-use efficiency in <i>Quercus robur</i> plants submitted to elevated CO <sub>2</sub> and drought. <i>Annales Des Sciences Forestieres</i> 53:431-446.
Polley et al., 1996	Polley, H. W., H. B. Johnson, H. S. Mayeux, D. A. Brown, and J. W. C. White. 1996. Leaf and plant water use efficiency of C-4 species grown at glacial to elevated CO <sub>2</sub> concentrations. <i>International Journal of Plant Sciences</i> 157:164-170.

References	
Possell and Hewitt, 2009	Possell, M. and C. N. Hewitt. 2009. Gas exchange and photosynthetic performance of the tropical tree <i>Acacia nigrescens</i> when grown in different CO <sub>2</sub> concentrations. <i>Planta</i> 229:837-846.
Prins et al., 2011	Prins, A., J. M. Mukubi, T. K. Pellny, P. J. Verrier, G. Beyene, M. S. Lopes, K. Emami, A. Treumann, C. Lelarge-Trouverie, G. Noctor, K. J. Kunert, P. Kerchev, and C. H. Foyer. 2011. Acclimation to high CO <sub>2</sub> in maize is related to water status and dependent on leaf rank. <i>Plant Cell and Environment</i> 34:314-331.
Qaderi and Reid, 2008	Qaderi, M. M. and D. M. Reid. 2008. Combined Effects of Temperature and Carbon Dioxide on Plant Growth and Subsequent Seed Germinability of <i>Silene noctiflora</i> . <i>International Journal of Plant Sciences</i> 169:1200-1209.
Qaderi and Reid, 2005	Qaderi, M. M. and D. M. Reid. 2005. Growth and physiological responses of canola ( <i>Brassica napus</i> ) to UV-B and CO <sub>2</sub> under controlled environment conditions. <i>Physiologia Plantarum</i> 125:247-259.
Qaderi et al., 2005	Qaderi, M. M., D. M. Reid, and E. C. Yeung. 2007. Morphological and physiological responses of canola ( <i>Brassica napus</i> ) siliques and seeds to UVB and CO <sub>2</sub> under controlled environment conditions. <i>Environmental and Experimental Botany</i> 60:428-437.
Retuerto and Woodward, 1993	Retuerto, R. and F. I. Woodward. 1993. The Influences of Increased CO <sub>2</sub> and Water-Supply on Growth, Biomass Allocation and Water-Use Efficiency of <i>Sinapis-Alba</i> L. Grown under Different Wind Speeds. <i>Oecologia</i> 94:415-427.
Robinson, 1999	Robinson, D. and J. P. Conroy. 1999. A possible plant-mediated feedback between elevated CO <sub>2</sub> , denitrification and the enhanced greenhouse effect. <i>Soil Biology &amp; Biochemistry</i> 31:43-53.
Robredo et al., 2007	Robredo, A., U. Perez-Lopez, H. S. de la Maza, B. Gonzalez-Moro, M. Lacuesta, A. Mena-Petite, and A. Munoz-Rueda. 2007. Elevated CO <sub>2</sub> alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. <i>Environmental and Experimental Botany</i> 59:252-263.
Robredo et al., 2007	Robredo, A., Perez-Lopez, U., de la Maza, H. S., Gonzalez-Moro, B., Lacuesta, M., Mena-Petite, A., & Munoz-Rueda, A. (2007). Elevated CO <sub>2</sub> alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. <i>Environmental and Experimental Botany</i> , 59(3), 252-263. doi: DOI 10.1016/j.envexpbot.2006.01.001
Roumet et al., 2000	Roumet, C., E. Garnier, H. Suzor, J. L. Salager, and J. Roy. 2000. Short and long-term responses of whole-plant gas exchange to elevated CO <sub>2</sub> in four herbaceous species. <i>Environmental and Experimental Botany</i> 43:155-169.
Sebastiani et al., 2002	Sebastiani, L., A. Minnoci, and R. Tognetti. 2002. Genotypic differences in the response to elevated CO <sub>2</sub> concentration of one-year-old olive cuttings ( <i>Olea europaea</i> L. cv. <i>Frantoio</i> and <i>Moraiolo</i> ). <i>Plant Biosystems</i> 136:199-207.
Seneweera et al., 1998	Seneweera, S. P., O. Ghannoum, and J. Conroy. 1998. High vapour pressure deficit and low soil water availability enhance shoot growth responses of a C-4 grass ( <i>Panicum coloratum</i> cv. <i>Bambatsi</i> ) to CO <sub>2</sub> enrichment. <i>Australian Journal of Plant Physiology</i> 25:287-292.
Sherwin et al., 2013	Sherwin, G. L., L. George, K. Kannangara, D. T. Tissue, and O. Ghannoum. 2013. Impact of industrial-age climate change on the relationship between water uptake and tissue nitrogen in eucalypt seedlings. <i>Functional Plant Biology</i> 40:201-212.
Sonnleitner et al., 2000	Sonnleitner, M. A., M. S. Gunthardt-Goerg, I. K. Bucher-Wallin, W. Attinger, S. Reis, and R. Schulin. 2001. Influence of soil type on the effects of elevated atmospheric CO <sub>2</sub> and N deposition on the water balance and growth of a young spruce and beech forest. <i>Water Air and Soil Pollution</i> 126:271-290.
Szente et al., 1998	Szente, K., Z. Nagy, and Z. Tuba. 1998. Enhanced water use efficiency in dry loess grassland species grown at elevated air CO <sub>2</sub> concentration. <i>Photosynthetica</i> 35:637-640.
Tezara et al., 2002	Tezara, W., V. Mitchell, S. P. Driscoll, and D. W. Lawlor. 2002. Effects of water deficit and its interaction with CO <sub>2</sub> supply on the biochemistry and physiology of photosynthesis in sunflower. <i>Journal of Experimental Botany</i> 53:1781-1791.
Thomas et al., 1994	Thomas, R. B., J. D. Lewis, and B. R. Strain. 1994. Effects of Leaf Nutrient Status on Photosynthetic Capacity in Loblolly-Pine ( <i>Pinus-Taeda</i> L.) Seedlings Grown in Elevated Atmospheric CO <sub>2</sub> . <i>Tree Physiology</i> 14:947-960.
Tognetti et al., 2001	Tognetti, R., L. Sebastiani, C. Vitagliano, A. Raschi, and A. Minnoci. 2001. Responses of two olive tree ( <i>Olea europaea</i> L.) cultivars to elevated CO <sub>2</sub> concentration in the field. <i>Photosynthetica</i> 39:403-410.
Townsend, 1993	Townsend, J. 1993. Effects of Elevated Carbon-Dioxide and Drought on the Growth and Physiology of Clonal Sitka Spruce Plants ( <i>Picea-Sitchensis</i> (Bong) Carr). <i>Tree Physiology</i> 13:389-399.
Tschaplinski et al., 1995	Tschaplinski, T. J., D. B. Stewart, P. J. Hanson, and R. J. Norby. 1995. Interactions between Drought and Elevated CO <sub>2</sub> on Growth and Gas-Exchange of Seedlings of 3 Deciduous Tree Species. <i>New Phytologist</i> 129:63-71.
Tuba et al., 1996	Tuba, Z., K. Szente, Z. Nagy, Z. Csintalan, and J. Koch. 1996. Responses of CO <sub>2</sub> assimilation, transpiration and water use efficiency to long-term elevated CO <sub>2</sub> in perennial C-3 xeric loess steppe species. <i>Journal of Plant Physiology</i> 148:356-361.
Tubiello et al., 1999	Tubiello, F. N., G. Lin, J. W. Druitt, and B. D. V. Marino. 1999. Ecosystem-level evapotranspiration and water-use efficiency in the desert biome of Biosphere 2. <i>Ecological Engineering</i> 13:263-271.
Vu and Allen, 2009	Vu, J. C. V. and L. H. Allen. 2009. Growth at elevated CO <sub>2</sub> delays the adverse effects of drought stress on leaf photosynthesis of the C-4 sugarcane. <i>Journal of Plant Physiology</i> 166:107-116.
Vu and Allen, 2009b	Vu, J. C. V. and L. H. Allen. 2009b. Stem juice production of the C-4 sugarcane ( <i>Saccharum officinarum</i> ) is enhanced by growth at double-ambient CO <sub>2</sub> and high temperature. <i>Journal of Plant Physiology</i> 166:1141-1151.
Vu et al., 2002	Vu, J. C. V., Y. C. Newman, L. H. Allen, M. Gallo-Meagher, and M. Q. Zhang. 2002. Photosynthetic acclimation of young sweet orange trees to elevated growth CO <sub>2</sub> and temperature. <i>Journal of Plant Physiology</i> 159:147-157.
Vu et al., 2006	Vu, J. C. V., L. H. Allen, and R. W. Gesch. 2006. Up-regulation of photosynthesis and sucrose metabolism enzymes in young expanding leaves of sugarcane under elevated growth CO <sub>2</sub> . <i>Plant Science</i> 171:123-131.
Vu, 2005	Vu, J. C. V. 2005. Acclimation of peanut ( <i>Arachis hypogaea</i> L.) leaf photosynthesis to elevated growth CO <sub>2</sub> and temperature. <i>Environmental and Experimental Botany</i> 53:85-95.
Wall et al., 2001	Wall, G. W., T. J. Brooks, R. Adam, A. B. Cousins, B. A. Kimball, P. J. Pinter, R. L. LaMorte, L. Triggs, M. J. Otman, S. W. Leavitt, A. D. Matthias, D. G. Williams, and A. N. Webber. 2001. Elevated atmospheric CO <sub>2</sub> improved Sorghum plant water status by ameliorating the adverse effects of drought. <i>New Phytologist</i> 152:231-248.
Wand et al., 1996	Wand, S. J. E., G. F. Midgley, and C. F. Musil. 1996. Growth, phenology and reproduction of an arid-environment winter ephemeral <i>Dimorphotheca pluvialis</i> in response to combined increases in CO <sub>2</sub> and UV-B radiation. <i>Environmental Pollution</i> 94:247-254.
Wang et al., 2012	Wang, R. G., S. X. Dai, S. R. Tang, S. Tian, Z. G. Song, X. F. Deng, Y. Z. Ding, X. J. Zou, Y. J. Zhao, and D. L. Smith. 2012. Growth, gas exchange, root morphology and cadmium uptake responses of poplars and willows grown on cadmium-contaminated soil to elevated CO <sub>2</sub> . <i>Environmental Earth Sciences</i> 67:1-13.
Warren J.	Warren JM, Poetzelberger E, Wulschleger SD, Thornton PE, Hasenauer H, Norby RJ. 2011. Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO2. <i>Ecohydrology</i> 4: 196–210.
Watling and Press, 1997	Watling, J. R. and M. C. Press. 1997. How is the relationship between the C-4 cereal <i>Sorghum bicolor</i> and the C-3 root hemi-parasites <i>Striga hermonthica</i> and <i>Striga asiatica</i> affected by elevated CO <sub>2</sub> ? <i>Plant Cell and Environment</i> 20:1292-1300.
Watling and Press, 2000	Watling, J. R. and M. C. Press. 2000. Infection with the parasitic angiosperm <i>Striga hermonthica</i> influences the response of the C-3 cereal <i>Oryza sativa</i> to elevated CO <sub>2</sub> . <i>Global Change Biology</i> 6:919-930.
Wayne et al., 1997	Wayne, P. M., E. G. Reekie, and F. A. Bazzaz. 1998. Elevated CO <sub>2</sub> ameliorates birch response to high temperature and frost stress: implications for modeling climate-induced geographic range shifts. <i>Oecologia</i> 114:335-342.
Wertin et al., 2010	Wertin, T. M., M. A. McGuire, and R. O. Teskey. 2010. The influence of elevated temperature, elevated atmospheric CO <sub>2</sub> concentration and water stress on net photosynthesis of loblolly pine ( <i>Pinus taeda</i> L.) at northern, central and southern sites in its native range. <i>Global Change Biology</i> 16:2089-2103.
Wu et al., 2004	Wu, D. X., G. X. Wang, Y. F. Bai, and J. X. Liao. 2004. Effects of elevated CO <sub>2</sub> concentration on growth, water use, yield and grain quality of wheat under two soil water levels. <i>Agriculture Ecosystems &amp; Environment</i> 104:493-507.



References	
Zaghdoud et al., 2013	Zaghdoud, C., C. Mota-Cadenas, M. Carvajal, B. Muries, A. Ferchichi, and M. D. Martinez-Ballesta. 2013. Elevated CO <sub>2</sub> alleviates negative effects of salinity on broccoli ( <i>Brassica oleracea</i> L. var <i>Italica</i> ) plants by modulating water balance through aquaporins abundance. <i>Environmental and Experimental Botany</i> 95:15-24.
Zhang and Dang, 2005	Zhang, S. R. and Q. L. Dang. 2005. Effects of soil temperature and elevated atmospheric CO <sub>2</sub> concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. <i>Tree Physiology</i> 25:523-531.
Zhang and Dang, 2006	Zhang, S. R. and Q. L. Dang. 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. <i>Tree Physiology</i> 26:1457-1467.
Zhang et al., 2008	Zhang, Y. B., B. L. Duan, Y. Z. Qiao, K. Y. Wang, H. Korpeläinen, and C. Y. Li. 2008. Leaf photosynthesis of <i>Betula albosinensis</i> seedlings as affected by elevated CO <sub>2</sub> and planting density. <i>Forest Ecology and Management</i> 255:1937-1944.
Zheng et al., 2010	Zheng, Y. R., Z. X. Xie, G. M. Rimmington, Y. J. Yu, Y. Gao, G. S. Zhou, P. An, X. J. Li, W. Tsuji, and H. Shimizu. 2010. Elevated CO <sub>2</sub> accelerates net assimilation rate and enhance growth of dominant shrub species in a sand dune in central Inner Mongolia. <i>Environmental and Experimental Botany</i> 68:31-36.
Zhou et al., 2013	Zhou, Y. M., X. J. Jiang, M. Schaub, X. J. Wang, J. Q. Han, S. J. Han, and M. H. Li. 2013. Ten-year exposure to elevated CO <sub>2</sub> increases stomatal number of <i>Pinus koraiensis</i> and <i>P. sylvestris</i> needles. <i>European Journal of Forest Research</i> 132:899-908.
Zhu et al., 1999	Zhu, J., G. Goldstein, and D. P. Bartholomew. 1999. Gas exchange and carbon isotope composition of <i>Ananas comosus</i> in response to elevated CO <sub>2</sub> and temperature. <i>Plant Cell and Environment</i> 22:999-1007.

## References (Table B2):

References	
Adam, et al., 2000	Adam, N. R., C. E. Owensby, and J. M. Ham. 2000. The effect of CO <sub>2</sub> enrichment on leaf photosynthetic rates and instantaneous water use efficiency of <i>Andropogon gerardii</i> in the tallgrass prairie. <i>Photosynthesis Research</i> 65:121-129.
Albert et al., 2011	Albert, K. R., H. Ro-Poulsen, T. N. Mikkelsen, A. Michelsen, L. Van der Linden, and C. Beier. 2011. Effects of elevated CO <sub>2</sub> , warming and drought episodes on plant carbon uptake in a temperate heath ecosystem are controlled by soil water status. <i>Plant Cell and Environment</i> 34:1207-1222.
Barton et al, 2011	Barton, C. V. M., R. A. Duursma, B. E. Medlyn, D. S. Ellsworth, D. Eamus, D. T. Tissue, M. A. Adams, J. Conroy, K. Y. Crous, M. Liberloo, M. Low, S. Linder, and R. E. McMurtrie. 2012. Effects of elevated atmospheric [CO <sub>2</sub> ] on instantaneous transpiration efficiency at leaf and canopy scales in <i>Eucalyptus saligna</i> . <i>Global Change Biology</i> 18:585-595.
Beerling, 1997	Beerling, D. J. 1997. Carbon isotope discrimination and stomatal responses of mature <i>Pinus sylvestris</i> L trees exposed in situ for three years to elevated CO <sub>2</sub> and temperature. <i>Acta Oecologica-International Journal of Ecology</i> 18:697-712.
Broadmeadow M.	Requested
Casella et al., 1996	Casella, E., J. F. Soussana, and P. Loiseau. 1996. Long-term effects of CO <sub>2</sub> enrichment and temperature increase on a temperate grass sward. 1. Productivity and water use. <i>Plant and Soil</i> 182:83-99.
Centritto et al., 1999a	Centritto, M., F. Magnani, H. S. J. Lee, and P. G. Jarvis. 1999b. Interactive effects of elevated [CO <sub>2</sub> ] and drought on cherry ( <i>Prunus avium</i> ) seedlings - II. Photosynthetic capacity and water relations. <i>New Phytologist</i> 141:141-153.
Conley et al., 2001	Conley, M. M., B. A. Kimball, T. J. Brooks, P. J. Pinter, D. J. Hunsaker, G. W. Wall, N. R. Adam, R. L. LaMorte, A. D. Matthias, T. L. Thompson, S. W. Leavitt, M. J. Otman, A. B. Cousins, and J. M. Triggs. 2001. CO <sub>2</sub> enrichment increases water-use efficiency in sorghum. <i>New Phytologist</i> 151:407-412.
Cui et al., 1993	Cui, M., P. M. Miller, and P. S. Nobel. 1993. CO <sub>2</sub> Exchange and Growth of the Crassulacean Acid Metabolism Plant <i>Opuntia-Ficus-Indica</i> under Elevated CO <sub>2</sub> in Open-Top Chambers. <i>Plant Physiology</i> 103:519-524.
Dixon et al., 1995	Dixon, M., D. Lethiec, and J. P. Garrec. 1995. The Growth and Gas-Exchange Response of Soil-Planted Norway Spruce [ <i>Picea-Abies</i> (L) <i>Karsf</i> ] and Red Oak ( <i>Quercus-Rubra</i> L) Exposed to Elevated CO <sub>2</sub> and to Naturally-Occurring Drought. <i>New Phytologist</i> 129:265-273.
Drake, 1992	Drake, B. G. 1992. A Field-Study of the Effects of Elevated CO <sub>2</sub> on Ecosystem Processes in a Chesapeake Bay Wetland. <i>Australian Journal of Botany</i> 40:579-595.
Ellsworth 1999	Ellsworth, D. S. 1999. CO <sub>2</sub> enrichment in a maturing pine forest: are CO <sub>2</sub> exchange and water status in the canopy affected? <i>Plant Cell and Environment</i> 22:461-472.
Ellsworth D.	Requested
Erbs et al., 2009	Erbs, M., J. Franzaring, P. Hög, and A. Fangmeier. 2009. Free-air CO <sub>2</sub> enrichment in a wheat-weed assembly - effects on water relations. <i>Basic and Applied Ecology</i> 10:358-367.
Fernandez et al., 2002	Fernandez, M. D., W. Tezara, E. Rengifo, and A. Herrera. 2002. Lack of downregulation of photosynthesis in a tropical root crop, cassava, grown under an elevated CO <sub>2</sub> concentration. <i>Functional Plant Biology</i> 29:805-814.
Fredeen and Field, 1995	Fredeen, A. L. and C. B. Field. 1995. Contrasting Leaf and Ecosystem CO <sub>2</sub> and H <sub>2</sub> O Exchange in <i>Avena-Fatua</i> Monoculture - Growth at Ambient and Elevated CO <sub>2</sub> . <i>Photosynthesis Research</i> 43:263-271.
Ge et al., 2012	Ge, Z. M., S. Kellomaki, X. Zhou, H. Peltola, K. Y. Wang, and P. J. Martikainen. 2012. Seasonal Physiological Responses and Biomass Growth in a Bioenergy Crop ( <i>Phalaris arundinacea</i> L.) Under Elevated Temperature and CO <sub>2</sub> , Subjected to Different Water Regimes in Boreal Conditions (vol 5, pg 637, 2012). <i>Bioenergy Research</i> 5:1067-1067.
Hao et al., 2013	Hao, X. Y., P. Li, Y. X. Feng, X. Han, J. Gao, E. D. Lin, and Y. H. Han. 2013. Effects of Fully Open-Air [CO <sub>2</sub> ] Elevation on Leaf Photosynthesis and Ultrastructure of <i>Isatis indigotica</i> Fort. <i>Plos One</i> 8.
Hileman et al., 1994	Hileman, D. R., G. Huluka, P. K. Kenjige, N. Sinha, N. C. Bhattacharya, P. K. Biswas, K. F. Lewin, J. Nagy, and G. R. Hendrey. 1994. Canopy Photosynthesis and Transpiration of Field-Grown Cotton Exposed to Free-Air CO <sub>2</sub> Enrichment (FACE) and Differential Irrigation. <i>Agricultural and Forest Meteorology</i> 70:189-207.
Ibrahim et al., 2010	Ibrahim, M. H., H. Z. E. Jaafar, M. H. Harun, and M. R. Yusop. 2010. Changes in growth and photosynthetic patterns of oil palm ( <i>Elaeis guineensis</i> Jacq.) seedlings exposed to short-term CO <sub>2</sub> enrichment in a closed top chamber. <i>Acta Physiologiae Plantarum</i> 32:305-313.
Kellomaki and Wang, 2001	Kellomaki, S. and K. Y. Wang. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. <i>Annals of Botany</i> 87:669-682.
Leverenz et al., 1999	Leverenz, J. W., D. Bruhn, and H. Saxe. 1999. Responses of two provenances of <i>Fagus sylvatica</i> seedlings to a combination of four temperature and two CO <sub>2</sub> treatments during their first growing season: gas exchange of leaves and roots. <i>New Phytologist</i> 144:437-454.
Lewis et al., 2002	Lewis, J. D., M. Lucash, D. M. Olszyk, and D. T. Tingey. 2002. Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure. <i>Plant Cell and Environment</i> 25:1411-1421.

References	
Liang and Maruyama, 1995	Liang, N. S. and K. Maruyama. 1995. Interactive effects of CO <sub>2</sub> enrichment and drought stress on gas exchange and water-use efficiency in <i>Alnus firma</i> . Environmental and Experimental Botany 35:353-361.
Llorens et al., 2009	Llorens, L., C. P. Osborne, and D. J. Beerling. 2009. Water-use responses of 'living fossil' conifers to CO <sub>2</sub> enrichment in a simulated Cretaceous polar environment. Annals of Botany 104:179-188.
Maroco et al., 1999	Maroco, J. P., G. E. Edwards, and M. S. B. Ku. 1999. Photosynthetic acclimation of maize to growth under elevated levels of carbon dioxide. Planta 210:115-125.
Nelson et al., 2004	Nelson, J. A., J. A. Morgan, D. R. LeCain, A. Mosier, D. G. Milchunas, and B. A. Parton. 2004. Elevated CO <sub>2</sub> increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado. Plant and Soil 259:169-179.
Norby and O'Neill, 1991	Norby, R. J. and E. G. O'Neill. 1991. Leaf-Area Compensation and Nutrient Interactions in CO <sub>2</sub> -Enriched Seedlings of Yellow-Poplar ( <i>Liriodendron-Tulipifera</i> L.). New Phytologist 117:515-528.
Picon et al., 1997	Picon, C., A. Ferhi, and J. M. Guehl. 1997. Concentration and delta C-13 of leaf carbohydrates in relation to gas exchange in <i>Quercus robur</i> under elevated CO <sub>2</sub> and drought. Journal of Experimental Botany 48:1547-1556.
Rey and Jarvis, 1997	Rey, A., & Jarvis, P. G. (1997). Growth Response of Young Birch Trees ( <i>Betula pendula</i> Roth.) After Four and a Half Years of CO <sub>2</sub> Exposure. Annals of Botany, 80(6), 809-816. doi: 10.1006/anbo.1997.0526
Rey and Jarvis, 1998	Rey, A. and P. G. Jarvis. 1998. Long-term photosynthetic acclimation to increased atmospheric CO <sub>2</sub> concentration in young birch ( <i>Betula pendula</i> ) trees. Tree Physiology 18:441-450.
Tausz-Posch et al., 2013	Tausz-Posch, S., R. M. Norton, S. Seneweera, G. J. Fitzgerald, and M. Tausz. 2013. Will intra-specific differences in transpiration efficiency in wheat be maintained in a high CO <sub>2</sub> world? A FACE study. Physiologia Plantarum 148:232-245.
Tissue et al., 1997	Tissue, D. T., R. B. Thomas, and B. R. Strain. 1997. Atmospheric CO <sub>2</sub> enrichment increases growth and photosynthesis of Pinus taeda: a 4 year experiment in the field. Plant Cell and Environment 20:1123-1134.
Tognetti et al., 1999	Tognetti, R., A. Longobucco, A. Raschi, F. Miglietta, and I. Fumagalli. 1999. Responses of two Populus clones to elevated atmospheric CO <sub>2</sub> concentration in the field. Annals of Forest Science 56:493-500.
Uddling et al., 2009	Uddling, J., Teclaw, R. M., Pregitzer, K. S., & Ellsworth, D. S. 2009. Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiology, 29(11), 1367-1380. doi: DOI 10.1093/treephys/tpp070
Warren et al., 2011	Warren JM, Poetzelberger E, Wullschlegel SD, Thornton PE, Hasenauer H, Norby RJ. 2011. Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO <sub>2</sub> . Ecohydrology 4: 196–210.