

On the variability of the ecosystem response to elevated atmospheric CO₂ across spatial and temporal scales at the Duke Forest FACE experiment

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Abstract

While the significance of elevated atmospheric CO₂ concentration on instantaneous leaf-level processes such as photosynthesis and transpiration is rarely disputed, its integrated effect at ecosystem level and at long-time scales remains a subject of debate. In part, the uncertainty stems from the inherent leaf-to-leaf variability in gas exchange rates. By combining 10 years of leaf gas exchange measurements collected during the Duke Forest Free Air CO₂ Enrichment (FACE) experiment and three different leaf-scale stomatal conductance models, the leaf-to-leaf variability in photosynthetic and stomatal conductance properties is examined. How this variability is then reflected in ecosystem water vapor and carbon dioxide fluxes is explored by scaling up the leaf-level process to the canopy using model calculations. The main results are: (a) the space-time variability of the photosynthesis and stomatal conductance response is considerable as expected. (b) Variability of the calculated leaf level fluxes is dependent on both the meteorological drivers and differences in leaf age, position within the canopy, nitrogen and CO₂ fertilization, which can be accommodated in model parameters. (c) Meteorological variability is playing the dominant role at short temporal scales while parameter variability is significant at longer temporal scales. (d) Leaf level results do not necessarily translate to similar ecosystem level responses due to indirect effects and other compensatory mechanisms related to long-term vegetation dynamics and ecosystem water balance.

Keywords: ecohydrological modeling, elevated CO₂, FACE, stomatal conductance model, spatio-temporal variability

Running Title: [Variability of ecosystem responses under elevated CO₂]

1 Introduction

Elevated atmospheric CO₂ (eCO₂) has short and long term consequences as well as direct and indirect effects on carbon and water fluxes and potentially on terrestrial biomass stocks. Specifically, the direct short term effect of eCO₂ is an increase in carbon assimilation at the leaf scale, and a reduction of stomatal conductance, as observed in the majority of species [Field *et al.*, 1995; Medlyn *et al.*, 2001; Long *et al.*, 2004; Ainsworth and Rogers, 2007; Lawson *et al.*, 2011]. Photosynthetic stimulation under eCO₂ is due to the enhanced carboxylation efficiency of Rubisco (*Ribulose - 1,5 - biphosphate carboxylase oxygenase*), and is more pronounced in C₃ than C₄ plants, which are carbon limited under current ambient concentrations of CO₂ (aCO₂) [Sage, 2004; Ainsworth and Rogers, 2007]. This leaf-level eCO₂ photosynthesis enhancement does not necessarily translate into similar increases in ecosystem carbon assimilation [Ainsworth and Long, 2005; Leakey *et al.*, 2009] or vegetation productivity and forest growth [Leuzinger *et al.*, 2011; Körner, 2013, 2015; Fatichi *et al.*, 2014]. In the long run, the increase in carbon assimilation is typically not followed by a respective increase in mineral nutrient availability, which ultimately can limit ecosystem net primary production (NPP) [Oren *et al.*, 2001; Luo *et al.*, 2004; Finzi *et al.*, 2006; Norby *et al.*, 2010]. Plants can also respond to this excess of carbon by down-regulating their photosynthetic machinery [Paul and Foyer, 2001; Crous *et al.*, 2008; Leakey *et al.*, 2009; Ellsworth *et al.*, 2012].

Elevated atmospheric CO₂ may cause a reduction in stomatal aperture that directly leads to decreased conductance to water vapor at the leaf scale [Field *et al.*, 1995; Medlyn *et al.*, 2001; Hetherington and Woodward, 2003]. At the canopy scale, an increase in leaf area associated with eCO₂ increases transpiring surface area but also within-canopy shading [Tor-ngern *et al.*, 2015]. The mechanistic description of the stomatal response to CO₂ and its signaling mechanism is an open question and may vary between species [Mott, 1990; Assmann, 1999; Brodribb *et al.*, 2009; Leakey *et al.*, 2009]. For a given leaf area and forcing, reduced stomatal conductance leads to (i) increased leaf temperatures due to a decrease in evaporative cooling, and (ii) more favorable soil water conditions [Rawson, 1992; Hyvönen *et al.*, 2007; De Kauwe *et al.*, 2013; Fatichi and Leuzinger, 2013; Keenan *et al.*, 2013]. Consequently, the interplay between plant responses to eCO₂ and hydrologic processes affects vegetation responses at the ecosystem scale that can trigger changes in long-term global vegetation dynamics and feedbacks on climate [Betts *et al.*, 2007; Zaehle *et al.*, 2007; Bonan, 2008; Sitch *et al.*, 2008; Friedlingstein *et al.*, 2014].

To unravel the mechanisms that affect the responses of ecosystems to eCO₂, several free air CO₂ enrichment experiments (FACE) have been established since the early 1990s [Owensby *et al.*, 1993; Lewin *et al.*, 1994; Zanetti *et al.*, 1996; Miglietta *et al.*, 1998, 2001; Hendrey *et al.*, 1999; Jordan *et al.*, 1999; Reich *et al.*, 2001; Edwards *et al.*, 2001; Norby *et al.*, 2001; Okada *et al.*, 2001; Bader *et al.*, 2013; Drake, 2014]. The

major achievement of those experiments in reference to laboratory or chamber-based CO₂ enrichment experiments is the quantification of the effect of carbon fertilization (such as transpiration changes, enhanced water use efficiency, growth stimulation, transience of plant responses), in natural settings where most of the interactions between plant physiological, micrometeorological, hydrological and edaphic processes occur (but see [Leuzinger *et al.*, 2015]).

To overcome the limited scope of inference of FACE experiments, and their short time span (typically less than a decade), terrestrial biosphere models have been used to assess the effects of eCO₂ on longer timescales [Cramer *et al.*, 1999; Krinner *et al.*, 2005; Sitch *et al.*, 2008; Scheiter *et al.*, 2013]. Those models integrate processes related to hydrology, plant physiology and forest demography, e.g., above- and belowground carbon dynamics, species competition for light, water and mineral resources. However, the theoretical potential of these models in quantifying responses of vegetation under eCO₂ and changing climate can be hampered by the limited knowledge of physiological processes as well as “boundary conditions” such as soil properties, vegetation composition and plant traits [Zaehle *et al.*, 2014; Körner, 2015; Medlyn *et al.*, 2015; Pappas *et al.*, 2015a, 2015b].

As these ecosystem models began confronting data from FACE experiments their predicted responses to eCO₂ appeared to deviate from measurements at several time scales. Such differences have been attributed to multi-species composition, local hydrological feedbacks, and feedbacks with leaf area, meteorological and land surface processes such as rainfall interception and vegetation aerodynamic coupling with the atmosphere [Curtis and Wang, 1998; Schäfer *et al.*, 2002; Nowak *et al.*, 2004; Friend and Kiang, 2005; Ainsworth and Rogers, 2007; Dermody *et al.*, 2007; Leakey *et al.*, 2009; Williams *et al.*, 2012; De Kauwe *et al.*, 2013; Zaehle *et al.*, 2014; Medlyn *et al.*, 2015]. What has not been explored is the variability in responses to eCO₂ even within uniform ecosystems, which is hereafter termed as ‘internal’ variability. This variability has both a spatial and a temporal component. The spatial variability of the responses within an ecosystem can reflect differences in physiological properties among individual leaves, canopy position or soil conditions, and the temporal variability of the responses relates primarily to exogenous meteorological drivers (on short time scales) and endogenous shifts in slowly evolving states (e.g., leaf acclimation to eCO₂, soil nitrogen depletion). The internal ecosystem variability can affect the assessment of whole ecosystem level responses to eCO₂ (or at minimum its statistical significance) because such internal variability must be integrated in space and time.

The scope of the current work is to investigate the role of this under-studied internal variability in the response of a pine plantation to eCO₂ at the leaf-level - and its up-scaled effect at the ecosystem scale. Using a combination of a decade of leaf gas-exchange and meteorological data re-analyzed for uniformity

at the Duke FACE site and stomatal and eco-hydrological modeling tools, we evaluate the variability of (a) the reduction of stomatal conductance and transpiration, (b) the increase in water use efficiency, and (c) the stimulation of carbon assimilation to eCO₂ at a range of temporal scales. The focus is on partitioning this leaf-level scale variability in simulated fluxes into its two main sources: (1) temporal, where the effects of meteorological drivers are explicitly considered; and (2) spatial, where the forest stand heterogeneity in the response of photosynthetic capacity and stomatal response to eCO₂ is examined. The proposed methodologies can be extended to variability of other factors such as those associated with edaphic processes. However, for carbon-water relations, the spatial and temporal variability in factors describing leaf-level gas exchange forms a logical starting point for all subsequent work. It is to be noted that while short-term temporal variability in leaf-level photosynthetic and stomatal conductance is considered in current Earth Systems Models, the importance of spatial variability and long-term trends in physiological properties have been mostly neglected.

2 Data and Methods

2.1 Data

The data used were collected at the Duke FACE experiment [Schlesinger *et al.*, 2006]. The site is located in Orange County, near Durham, North Carolina, USA within the Duke Forest (79.09W, 35.98N, 168 m a.s.l.). The forest was established as a loblolly pine (*Pinus taeda*) plantation from 3-year old seedlings [Pritchard *et al.*, 2008] in 1983 after a clear cut in 1982. During the experiment, several deciduous understory species were naturally established (e.g. *Liquidambar styraciflua*, *Acer rubrum*, *Ulmus alata*, *Cercis Canadensis*, *Cornus florida*, *Liriodendron tulipifera*) [Schlesinger *et al.*, 2006; Springer and Thomas, 2007]. The soil in the area is a silt loam with an impermeable clay pan at about 30 cm [Oishi *et al.*, 2010]. Annual precipitation is about 1100 mm uniformly distributed across seasons and the mean annual temperature is 15°C. A summary of the diurnal and seasonal fluctuations of meteorological data is given in Figure 1. CO₂ fumigation was initiated in 1994 in a prototype plot and the full FACE site became operational in 1996. In total, eight 30-m diameter circular plots were constructed (four aCO₂; four eCO₂) [Feng *et al.*, 2010]. Since 2005, the rings were split into quadrants and N fertilization (11.2 g of N m⁻²yr⁻¹ as ammonium nitrate) was applied to half of them. The Duke Forest FACE experiment was terminated in 2010 and the site was de-commissioned.

During the experiment, a large number of leaf gas exchange measurements were conducted [Katul *et al.*, 2000; Crous and Ellsworth, 2004; Crous *et al.*, 2008; Maier *et al.*, 2008; Palmroth *et al.*, 2013] and collected in a common database [Ellsworth *et al.*, 2012]. Here, only data on loblolly pine are used. While

details on the sampling and measurement protocols can be found elsewhere (see citations above) the framework common to all studies is briefly described as follows. All measurements were carried out on intact leaves or leaves on cut branches (re-cut under water) using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NB, USA) equipped with a standard leaf chamber. Leaves were sampled from different levels inside the canopy (upper canopy sun-exposed and lower canopy shaded leaves), for all the treatment combinations (CO_2 , N fertilizations) and for different leaf age classes (current-year [new leaves]/1-year-old [old leaves]). The dataset used here includes 485 curves, i.e. the responses of measured CO_2 exchange (A_n) and transpiration rates (T) to variation in (inferred) internal CO_2 concentration or c_i (hereafter referred to as $A_n - c_i$ curves). The measurements were unevenly distributed between different treatment combinations and leaf age or level classes (Table 1). Meteorological and eddy-covariance CO_2 and water vapor flux data were available for ambient conditions and were collected on a 22-m tall flux tower (Fluxnet site US-Dk3) located within the same pine plantation from 1998 to 2008 [Oishi *et al.*, 2010; Paschalis *et al.*, 2015]. Due to the close proximity of all replicate plots of the Duke-FACE site, the same meteorological forcing is used for all of them. No major data gaps exist in the record and variable dependent thresholds were used for quality control and outlier elimination in the records.

2.2 Models

The models used to describe leaf level gas-exchange and ecosystem level processes are briefly described. At the leaf level, the standard biochemical demand model for C3 photosynthesis and three different models describing the responses of stomatal conductance to environmental drivers and atmospheric CO_2 concentration are used. The outcome of these models is then introduced into a mechanistic process-based eco-hydrological model that resolves the essential hydrological and plant physiological processes at the ecosystem (or stand-level) scale.

2.2.1 Leaf Level Models

Net photosynthesis (A_n) is modeled according to the conventional biochemical demand formulation [Farquhar *et al.*, 1980] taking into account subsequent modifications introduced in a number of studies [Collatz *et al.*, 1991; Dai *et al.*, 2004; Kattge and Knorr, 2007; Bonan *et al.*, 2011]. Here, $A_n = A_g - R_d$, where A_g is gross photosynthesis rate and R_d is leaf maintenance respiration (i.e. mitochondrial respiration). The A_g is modeled as a function of the three limiting factors of photosynthesis: the Rubisco limitation (J_c), the light limitation (J_e) and the capacity of the leaf to export and utilize the products of photosynthesis (J_s). The term A_g depends on the biochemical parameters of photosynthesis, such as the intrinsic quantum efficiency (ϕ), the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}), and environmental conditions such as the absorbed photosynthetically

active radiation ($APAR$), the leaf temperature (T_s) and the concentration of CO_2 in the leaf intercellular space (c_i), or more precisely inside the chloroplasts (c_c) if a mesophyll conductance term is added. The term R_d is assumed to depend solely on V_{cmax} and leaf temperature. All the relevant equations used in this study can be found in full detail in [Fatichi, 2010; Fatichi et al., 2012].

Stomata respond to environmental factors such as relative humidity (RH) or vapor pressure deficit (D), atmospheric concentration of CO_2 (c_a) and soil moisture. The precise mechanisms that drive the regulation of guard cell turgor pressure, and thus the stomatal aperture, are still a matter of open research [e.g., Buckley, 2005; Buckley and Mott, 2013; McAdam and Brodribb, 2014]. The mechanisms of the chemical/hormone signaling for stomatal regulation are rather complex [Tardieu and Davies, 1993; Comstock, 2002; Kim et al., 2010; Fatichi et al., 2016] and a commonly accepted mechanistic model for guard cell function does not exist. Instead, several semi-empirical formulations that quantify the response of stomatal conductance to changes in environmental conditions exist and are used in climate models. The three most common are the Ball-Woodrow-Berry (or Ball-Berry) model [Ball et al., 1987], the Leuning model [Leuning, 1995], and models based on optimality principles that compute stomatal conductance by maximizing carbon gain for a unit of water loss [Cowan and Farquhar, 1977; Beringer et al., 1996; Mäkelä et al., 1996; Katul et al., 2010; Medlyn et al., 2011].

The Ball-Berry model is defined as:

$$g_s = m_b \frac{A_n RH}{c_s} + g_0 \quad \text{Eq 1}$$

where m_b is a model parameter, c_s is the molar fraction of CO_2 at the leaf surface, and g_0 is a residual conductance, commonly related to imperfect stomatal closure and the cuticular conductance of leaves.

The Leuning model is defined as:

$$g_s = m_l \frac{A_n P_{atm}}{(pc_s - \Gamma)(1 - D/D_0)} + g_0 \quad \text{Eq 2}$$

where m_l is a model parameter, P_{atm} is the atmospheric pressure, pc_s is the partial pressure of CO_2 at the leaf surface, Γ is the photosynthetic CO_2 compensation point expressed as a partial pressure, and D_0 is a second model parameter rescaling the sensitivity of stomata to vapor pressure deficit. It has been argued that the partial pressure of CO_2 in the intercellular air space (pc_i) is a more reasonable assumption than at the leaf surface [Mott and Morr, 1988; Assmann, 1999]. Here, the formulation using pc_s is retained for comparison with previous results obtained for the same site [Katul et al., 2010]. However, we repeated the entire analysis using pc_i (not reported here), and the results were virtually the same due to the strong correlation between pc_i and pc_s .

The last model considered is based on the maximization problem

$$\operatorname{argmax}_{g_s} \left(\int A_n dt - \lambda \int T dt \right) \quad \text{Eq 3}$$

where T is the transpiration rate and λ is a model parameter. When water availability does not vary appreciably over the integration period, the integral operator is no longer necessary and the maximization over the entire integration period can be achieved by maximizing the instantaneous Hamiltonian $A_n - \lambda T$ as shown in *Manzoni et al.* [2013]. The optimization problem states that stomatal conductance adjusts such that there is maximum carbon gain for a given water loss. The parameter λ is the Lagrange multiplier of the optimization problem and can be shown to be equivalent to the marginal water use efficiency $\lambda = (\partial A_n / \partial g_s) / (\partial T / \partial g_s)$ [Manzoni et al., 2011b]. For the constant (or slowly evolving with respect to g_s) λ , analytical relations between g_s and leaf metabolism, environmental conditions and c_s can be derived [Katul et al., 2010; Manzoni et al., 2011b; Medlyn et al., 2011; Vico et al., 2013]. All of them predict a linear or quasi-linearized scaling of $g_s \propto A_n c_s^{-1} D^{-1/2}$. Other optimality models, such as those that assume leaves minimize the summed unit costs of transpiration and carboxylation predict similar responses [Prentice et al., 2014] and are not treated here. However, to include all the limitations such as J_s and ensure that the instantaneous Hamiltonian remains positive for all environmental conditions, especially when photosynthetic limitations shift, the maximization problem is solved numerically using a steepest descent algorithm (see also [Bonan et al., 2014]).

2.2.2 Ecosystem Model

To scale up the effects of $e\text{CO}_2$ from leaf to ecosystem, the ecohydrological/ecosystem model “Tethys-Chloris” (T&C hereinafter) [Fatichi et al., 2012; Fatichi and Leuzinger, 2013; Fatichi and Ivanov, 2014; Paschalis et al., 2015, 2016; Pappas et al., 2016] is employed. T&C resolves the energy and water balance in the soil and at the land surface and models ecosystem vegetation dynamics. Carbon pools, energy exchanges, and water stores and fluxes are fully prognostic in the model.

Specifically, the model is forced with hourly meteorological variables (precipitation, temperature, incoming shortwave/longwave radiation, wind speed, atmospheric pressure and relative humidity) and resolves the radiation transfer through the canopy, the water fluxes in the soil and the canopy (interception, throughfall, soil water flow, evaporation/sublimation and transpiration) and the carbon fluxes (photosynthesis and respiration) at the canopy level. The model is fully mechanistic without adding a large degree of abstraction, and most processes are modeled according to physically based formulations. The carbon and water cycles are linked through stomatal regulations and the limitation that available soil water imposes on carbon assimilation. The temporal dynamics of vegetation (e.g. leaf area index (LAI) evolution, biomass evolution)

are modeled by balancing the carbon gain (photosynthesis) and losses (respiration, tissue turnover). Vegetation dynamics are modeled using the concept of plant functional types (PFTs) or species-specific parameters. Vegetation is conceptualized as a series of carbon pools (leaves, fine roots, living sapwood, non-structural carbohydrates, fruits, heartwood, dead standing leaves) that evolve in time by partitioning the net primary production based on specific (but dynamic) allometric rules and phenology.

T&C uses the same leaf photosynthesis model earlier described. Stomatal conductance is modeled based on the Leuning formulation. A detailed model description and its entire mathematical formulation can be found elsewhere [Fatichi, 2010; Fatichi *et al.*, 2012] and is not repeated here.

2.3 Data Analysis

Parameters regulating the biochemical demand of photosynthesis were estimated using data from the leaf gas exchange measurements. Only the parameters V_{cmax} and J_{max} were estimated, while the rest (e.g., intrinsic quantum efficiency, temperature response in terms of activation energies and entropy factors, Michaelis-Menten coefficients) were considered constant among all measured $A_n - c_i$ curves and identical to the study by Paschalis *et al.*, [2015] (intrinsic quantum efficiency $\varepsilon = 0.081 \mu\text{mol CO}_2/\mu\text{mol photons}$, activation energy $H_a = 0.649 \text{ kJ/mol}$, entropy factor $\Delta S = 72 \text{ kJ/molK}$, Michaelis-Menten coefficients are related to leaf temperature as in Fatichi, [2010]). This is a reasonable assumption since the degree of variability of V_{cmax} and J_{max} is expected to be much larger in comparison to the remaining parameters [Miao *et al.*, 2009]. Several methods for the estimation of V_{cmax} and J_{max} from $A_n - c_i$ curves exist [Miao *et al.*, 2009]. We chose to estimate the parameters by minimizing the sum of squared deviations $\sum (A_n^m(V_{cmax}, J_{max}, c_i) - A_n^o(c_i))^2$, where A_n^m is the modeled net photosynthesis for an intercellular CO_2 concentration c_i , and A_n^o is the observed net photosynthesis from the gas exchange measurements. With this methodology, all measurements are used in the parameter estimation, instead of the common technique of estimating V_{cmax} from the strictly Rubisco limited region of the curve, and J_{max} from the strictly light limited region. Note that the region where co-limitations occur is ignored with this common technique. The curves for which the goodness of fit $R^2 < 0.9$, or the ratio $J_{max}/V_{cmax} > 4$ were excluded from the analysis. The results were grouped based on the treatments (CO_2/N fertilization), leaf age class and leaf position inside the canopy (mainly height above the forest floor). Every $A_n - c_i$ curve was considered independent. This assumption is reasonable given the large variability of photosynthetic capacity (i.e. V_{cmax} and J_{max}) among leaves within a tree and at a given height above the forest floor [Luoma, 1997; Niinemets, 2007]. Inter-annual variability, which might be related to transient plant responses to the progressive treatment effect, or seasonal differences were not computed [Crous and Ellsworth, 2004] in this evaluation.

The leaf exchange measurements were also used for the estimation of the parameters of the stomatal responses. For every group, the parameters of the Ball-Berry and the Leuning model are estimated using a least squares approach fitting the observations. Only values of $200 < c_s < 700$ [ppm] were used since they provided a much better fit to the data (Figure 2). Very high and low values of c_s resulted in outliers. To compute the variability within each group, a bootstrap sampling (i.e. random sampling with replacement) of 100 realizations was used. The parameter value λ of the optimality model was computed numerically approximating the marginal water use efficiency $\lambda = (\partial A_n / \partial g_s) / (\partial T / \partial g_s)$. For every gas exchange curve, the λ was numerical determined using a second-order accurate central differencing given as $\lambda = (A_n(g_s + \delta, \theta) - A_n(g_s - \delta, \theta)) / (2\delta a D)$, where $A_n(g_s, \theta)$ is the estimated net assimilation rate for the photosynthesis parameters function of g_s and of a given set of environmental variables $\theta = [T_a, APAR, c_s, D]$ and $a = 1.64$ is the ratio between the molecular diffusivities of water vapor and CO₂. The D is expressed in [Pa] and not as a molecular ratio for unit compatibility between this study and others [e.g., Katul *et al.*, 2010] i.e. λ in [mol mol⁻¹ Pa⁻¹]. The δ is a fraction set to 1% of the measured stomatal conductance g_s . Since an estimate of λ for every measurement can be calculated, there is no need for bootstrap sampling in this case. In all of the cases reported, the residual conductance was neglected ($g_0 = 0$). In the Leuning model, a constant value of the parameter $D_0 = 1000$ Pa was used for all cases. In the supplementary material, additional results that investigated the importance of the marginal conductance g_0 and also the mesophyll conductance g_{mes} (i.e. conductance to CO₂ between the intercellular space and the center of photosynthetic site in the chloroplasts) are provided.

To assess the differences between the mean values of each of the photosynthesis and stomata-related parameters ($V_{cmax}, J_{max}, m_l, m_b, \lambda$) according to the CO₂, N fertilization treatments, the leaf age class and the level of the samples within the canopy, an ANOVA was performed taking into account all the interactions. Covariation of the dependent variables ($V_{cmax}, J_{max}, m_l, m_b, \lambda$) of the ANOVA was neglected.

2.4 Simulations

The first set of simulations includes the quantification of the general patterns of responses for all three stomatal conductance formulations when they are coupled with the photosynthesis model. The second set of simulations is used for the quantification of the relative importance of the variability of model parameters and meteorological drivers on the leaf and subsequently on ecosystem responses to eCO₂.

2.4.1 Stomatal Conductance Model Responses to Meteorological Forcing

The effects of eCO₂ on A_n , T , and water use efficiency (defined as $WUE = A_n / T$) for various combinations of environmental forcing parameters summarized as vector θ and for the three stomatal conductance models is analyzed here. The estimation of these responses is not trivial and depends on (a)

the responses of the stomata to the metabolic and environmental conditions and (b) on the coupling between the leaf surface and the atmosphere. For this reason, the response patterns of each stomatal conductance model are first investigated separately. How these responses affect the leaf level energy balance and ultimately A_n , T , and WUE is then considered. In this first set of simulations, a full solution of the leaf energy balance coupled with the leaf biochemical module for photosynthesis and stomatal responses to environmental factors is carried out. The solution consists of an iterative scheme (a fixed point iteration for T_s), which can be computationally demanding.

The value of A_n depends on absorbed PAR , the temperature of the leaf surface (T_s) and the CO_2 concentration at the site of photosynthesis (c_c). For all the modeling experiments considered here the CO_2 mesophyll conductance between the intercellular space (c_i) and the chloroplasts (c_c) is considered large and thus $c_c \simeq c_i$, even though there is evidence against this argument [Niinemets *et al.*, 2009; Flexas *et al.*, 2012; 2015]. Including a constant mesophyll conductance would change the estimate of the biochemical parameters (Section 2.3) but not the overall behavior of the models. The concentration c_i depends on the strength of the coupling between the leaf interior and the atmosphere, which is determined by stomatal conductance and T_s . The latter depends on the environmental forcing, the stomatal conductance (that affects leaf evaporative cooling), and the geometric characteristics of the leaves [Vogel, 2009; Schymanski *et al.*, 2013; Huang *et al.*, 2015a] including leaf micro-roughness [Huang *et al.*, 2015b]. Similar arguments can be made for T and WUE . It clearly emerges that A_n , T , and WUE are affected by environmental drivers in a nonlinear manner, and the choice of stomatal conductance model alone can be of major significance in determining their values and variations. The responses of stomatal conductance for several combinations of environmental factors are obtained by simultaneously solving the leaf surface energy balance and the CO_2 flux from the atmosphere to the leaf interior. Moreover, λ , which depends on A_n , g_s and T , could also be determined for all models as a complementary output. In the main text, the responses of λ and g_s to the environmental forcing for all three models are presented in a condensed form, while in the supplementary material, details concerning the responses of g_s , T , WUE , and also c_i/c_a to the environmental forcing are featured.

2.4.2 Leaf-level Monte Carlo Simulations

To assess the effect of meteorological and parameter variability on the responses of leaves to eCO_2 , a Monte Carlo experiment was constructed. First, for each group representing a different combination of treatments, leaf age, and canopy level, a random parameter vector \mathbf{u} containing the photosynthetic and stomatal conductance parameters was drawn from the fitted distributions to the measured values. The distributions were assumed to be Gaussian with mean values and variances equal to the measured values, and the cross-correlation between each parameter was neglected. Second, the leaf-level model for photosynthesis coupled

with the models for stomatal conductance was forced with the observed meteorological drivers for two levels of atmospheric CO₂ concentrations (aCO₂: 380 ppm; eCO₂: 580 ppm) and for each of two light environments: the upper canopy with sun-exposed leaves, and the lower canopy with shade-acclimated leaves. For the upper canopy, PAR was set equal to the observed values above the canopy, and for the bottom of the canopy, a reduction of PAR was generated assuming a Beer–Lambert light attenuation $PAR_{shd} = PAR_{sun} \exp(-k \text{ LAI})$. In the leaf-level numerical experiments, values of LAI=4 and $k=0.5$ were used as representative of the Duke pine forest [McCarthy *et al.*, 2007]. In total, 30 different realizations were sampled. In this simulation setup, only well coupled atmospheric conditions were taken into account (i.e. $T_s = T_a$) for reasons that will be explained more thoroughly in the result section.

Three different statistics were used to quantify the effect of meteorological and parameter variability on the uncertainty of the responses to eCO₂:

$$(a) s_1 = \int_0^\tau A_n^e dt / \int_0^\tau A_n^a dt,$$

$$(b) s_2 = \int_0^\tau T^e dt / \int_0^\tau T^a dt, \text{ and}$$

$$(c) s_3 = WUE^e(\tau) / WUE^a(\tau),$$

where $WUE^a(\tau) = \int_0^\tau A_n^a dt / \int_0^\tau T^a dt$, $WUE^e(\tau) = \int_0^\tau A_n^e dt / \int_0^\tau T^e dt$, $A_n^a(A_n^e)$ is the net CO₂ assimilation under ambient(elevated) CO₂ and $T^a(T^e)$ is the transpiration under ambient(elevated) CO₂. To analyze the effect of temporal scale on the leaf-level responses to eCO₂, three time scales ($\tau=1$ hour, 1 day, and 1 year) were used. The terms s_1 , s_2 and s_3 represent the eCO₂ effect on net CO₂ assimilation, transpiration and WUE , respectively.

For partitioning the variability of the responses to leaf-scale parameter and meteorological variability, a procedure similar in concept to the one-way ANOVA was used. The total variability of the responses of a statistic s is defined by the distribution of s , taking into account all the realizations (i.e. their union \cup) of the stomatal and photosynthesis parameters, i.e. the distribution of

$$S_{TOT} = \bigcup \{s^1(t), s^2(t), \dots, s^n(t)\},$$

for all n realizations and for every time step t , where the meteorological conditions are different. The number of time steps depends on the length of the meteorological records and on the time scale being

examined. In every realization (i from 1 to n), the model parameters differ based on the Monte Carlo sampling procedure. Every time scale ($\tau=1$ hour, 1 day, and 1 year) and every statistic (s_1, s_2, s_3) are examined separately. The variability due to the model parameters can be estimated by the distribution of the mean values of the responses of the statistic s , averaging all time steps t , for each realization i , i.e.

$$S_{PAR} = [\overline{s^1}, \overline{s^2}, \dots, \overline{s^n}],$$

where $\overline{s^i} = \frac{1}{m} \sum_{t=1}^m s^i(t)$. The variability introduced by the meteorological conditions can be estimated by the distribution of

$$S_{ENV} = \bigcup \{s^1(t) - \overline{s^1} + \overline{s^{all}}, s^2(t) - \overline{s^2} + \overline{s^{all}}, \dots, s^n(t) - \overline{s^n} + \overline{s^{all}}\},$$

where $\overline{s^{all}} = \frac{1}{m} \frac{1}{n} \sum_{t=1}^m \sum_{i=1}^n s^i(t)$. In this study the standard deviation of the sets $S_{TOT}, S_{PAR}, S_{ENV}$ was used as a metric of their variability.

2.4.3 Ecosystem Level Simulations

The purpose of this simulation is to investigate how the observed variability at the leaf scale (only) manifests itself at ecosystem scale, taking into account the major eco-hydrological feedbacks.

Given the relatively high computational demand of T&C, only margin or end-member cases were sampled. Specifically, two values of V_{cmax} were selected for the upper canopy representing approximately the 25% and 75% percentiles of the measured V_{cmax} for all the cases of the upper canopy estimates (i.e. the percentiles derived from the pool of estimated V_{cmax} values, lumping together all subcases of nitrogen treatment, carbon fertilization and age class). A differentiation in canopy-level class was not prescribed in those simulations, because T&C itself simulates a decline of V_{cmax} within the canopy proportional to a nitrogen decay coefficient. The leaf age class was also excluded in this experiment. Similarly, two values of the Leuning model parameter were sampled such that they represent approximately the 25% and 75% percentiles of the observed values between all groups (i.e. the percentiles derived from the pool of parameter estimates lumping together all subcases of nitrogen treatment, carbon fertilization, age class and level within the canopy). Moreover, in the ecosystem level simulations, both overstory (pines) and understory (deciduous hardwood species) vegetation are explicitly simulated to mimic the real ecosystem. For the understory only, a single parametrization throughout the simulations was used, given its minor contribution to the overall fluxes. The model parameter values for the deciduous understory species were taken from *Paschalis et al.*, [2015]. The set of statistical analyses described above are used to quantify the effects of variability in the parameter values and the environmental drivers at the ecosystem scale.

3 Results

3.1 Leaf Level

3.1.1 Variability in V_{cmax} , J_{max} and Stomatal Conductance Within Canopy and Across Treatments

The variability in V_{cmax} and J_{max} (scaled to 25°C) within each group (canopy level/leaf age/treatment) is large (Figure 3). This reflects the natural spatial variability, but also may integrate variability due to measurement errors, and the transient behavior of long-term acclimations given that inter-annual variability of the measurements was not explicitly taken into account. Despite the large within-group variability, ANOVA suggests that both V_{cmax} and J_{max} of current-year needles decrease with decreasing light availability within the canopy (low p-values of L and A treatments in Table 2). Both parameters depend on nitrogen content per unit leaf area, which decreases with light availability within the canopy [Niinemets *et al.*, 1998, 2015; Grassi and Bagnaresi, 2001; Warren *et al.*, 2003]. Compared to current-year foliage, 1-year-old needles, in most cases, have significantly lower V_{cmax} and J_{max} . Finally, V_{cmax} and J_{max} appear non-responsive to eCO₂ (Table 2). This contradicts earlier arguments that a down-regulation of the photosynthetic machinery of plants takes place under long-term exposure to eCO₂ [e.g. Medlyn *et al.*, 1999]. At the Duke FACE site, previous research suggested that plant structural adjustments occur after long exposure to eCO₂, such as changes in the relation between leaf nitrogen concentration and photosynthetic capacity or V_{cmax} , particularly in 1-year-old needles [Crous *et al.*, 2008; Maier *et al.*, 2008]. However, the non-responsiveness of V_{cmax} and J_{max} has also been documented by Crous & Ellsworth [2004] who identified a lack of a strong, statistically significant reduction of V_{cmax} and J_{max} at the Duke FACE. It should be highlighted that this lack of response might be influenced by the transient long-term acclimation to eCO₂ which was not taken into account (or at least can partially mask eCO₂ effect). Also, the potential seasonal fluctuations of V_{cmax} at 25 C, were not taken into account [Zhang *et al.*, 2014b], even though there is contradictory evidence whether V_{cmax} varies strongly on a seasonal basis in the Duke Forest [Ellsworth, 2000; Juang *et al.*, 2006; Ellsworth *et al.*, 2012].

The parameters of the stomatal responses to environmental factors were statistically different among the various groups (Figure 4) as shown from the low p-values in ANOVA results in Table 2. However, these differences are difficult to interpret as they are influenced by two major factors. First, there is considerable uncertainty in the parameter estimation itself (Figure 2) [see also Yu *et al.*, 2004; Medlyn *et al.*, 2011]. Second, all the results were obtained assuming that the marginal or residual conductance was equal to zero, $g_0 = 0$, and different patterns are obtained when this assumption is removed as theoretically considered elsewhere [Manzoni *et al.*, 2011b]. For these reasons, we refrain from providing detailed explanations regarding the among-groups differences in the values of these parameters, especially when patterns are not

univocal. The results of the parameter estimation of all three g_s models (with $g_0 = 0$) are used in the following only to assess their variability. It is to be noted that the degree of variability between the g_s model parameter estimation when accounting for g_0 is comparable to those with $g_0 = 0$, despite the fact that parameter values are different (analysis not shown here).

3.1.2 Differences in Stomatal Conductance Model Behavior

The response of stomatal conductance as predicted by all three g_s models (Eqs 1-3) depends on the environmental factors as shown in Figure 5 and in the Supplementary material. The key characteristic of all the responses are: (a) a bell-shaped response in g_s to temperature; (b) a steep increase in g_s with increased absorbed PAR , reaching a plateau for high irradiance; (c) a minimal influence of wind speed; (d) a positive relation between g_s and relative humidity; and (e) a reduction in g_s under eCO_2 . The first two observations can be fully explained by the response of A_n to T_s and PAR , and the dependence of g_s on A_n . The fact that wind speed (W_s) has minimal influence can be explained by the leaf shape (needles) that does not allow the development of a thick laminar boundary layer [Huang *et al.*, 2015a]. For this reason, only well-coupled conditions were assumed in the subsequent Monte Carlo simulations.

The major functional difference between all the models is the response of g_s to RH . The Ball-Berry model prescribes a linear relation between those variables (Eq. 1), in contrast to the Leuning model that expresses g_s as a function of D . The g_s response to D is not a priori imposed and is an emergent outcome of the stomatal optimization. These two models result in a nonlinear dependence of g_s on RH . This dependence of g_s on RH results in similar responses of A_n , T , c_i/c_a and WUE between the optimality and Leuning model formulations, but significant differences in comparison to the Ball-Berry model (Supplementary material). For this reason, in the following, only two models (Ball-Berry and Leuning) are used for further analysis, given their current popularity in Earth System Models and the functional similarity between the Leuning model and the optimality model (Figure 5).

Another difference between the three models is the estimated value of the marginal water use efficiency λ . Roughly, λ is interpreted as the cost of water loss from stomata in units of carbon. It is of interest here because it bridges the water and carbon economies of plants at the leaf scale. As λ increases, carbon becomes easier to acquire, “cheaper”, and at the same time water loss becomes more “expensive” [Katul *et al.*, 2009, 2010; Manzoni *et al.*, 2011a, 2011b]. By definition, λ in the optimality model is constant on time scales over which stomatal aperture fluctuates (usually sub-daily). In the Ball-Berry and Leuning models, a value of λ can be computed and depends on both the environmental drivers and the level of CO_2 enrichment. The λ patterns are substantially different between the models (Figure 6). The two g_s models predict a relatively steady λ for a wide range of relative humidity conditions, suggesting close to optimal

behavior. This range of relative humidity ($0.2 < RH < 0.9$) corresponds to the most common values expected for temperate and continental climates. However, discrepancies in predicted λ between the Leuning and the Ball-Berry models appear at the RH extremes. In general, the Ball-Berry model predicts a high value of λ for low RH , which suggest that this model assigns a “high” cost of water in a water-carbon cost-benefit perspective, and for this reason, the predicted stomatal conductance for this model is much lower (Supplementary material) than the other two g_s models at low RH . In contrast, the Leuning g_s model predicts a high value of λ at high RH , suggesting lower than optimal stomatal conductance at low D (Figure 5). Interestingly, both g_s models predict a higher value of λ with increased temperature. Finally, the λ computed from the Leuning modeled is more sensitive than its Ball-Berry counterpart in terms of $|\partial\lambda/\partial c_a|$.

3.1.3 Sensitivity to spatial and temporal variability

The key results from the leaf-level simulations are: (a) at short temporal scales (e.g. hourly) the main source of the variability in the responses to eCO_2 is the environmental forcing; (b) as temporal scales increase, the influence of variability in model parameters becomes predominant; and (c) the choice of the stomatal conductance model can result in appreciable differences in the mean values of the responses of A_n , WUE , T and their variability (Table 3, Figure 7). Here, the results of the leaf level variability of the responses to eCO_2 for all combinations of treatments are featured (Table 3, Figure 7), whereas in the Supplementary material, the case-by-case analysis is presented. As a metric of the variability of the responses to eCO_2 , we chose the standard deviation of the sets S_{TOT} , S_{PAR} and S_{ENV} (Table 3). In Figure 7, the variability of the responses to eCO_2 can be interpreted by the range of the respective box plots.

Specifically, the total variability of the net CO_2 assimilation effect at the hourly time scale varies from 1-1.7 for the Ball-Berry g_s model and from 1-1.5 for the Leuning g_s model. For this estimation, only the hours when A_n^e and $A_n^a > 1 \mu mol CO_2 m^{-2} s^{-1}$ were used. This variability can be explained almost entirely by the environmental forcing. Furthermore, it is trivial to show that the ratio of the instantaneous water use efficiency (WUE) in eCO_2 and aCO_2 for the Ball-Berry model equals the ratio of the concentrations $[eCO_2]/[aCO_2]$ and is independent of model parameters and environmental forcing (for the Ball-Berry model and $g_0 = 0$, the instantaneous $WUE = A_n/g_s D = c_s/m_b RH$ and thus $WUE^e/WUE^a = [eCO_2]/[aCO_2]$). For the Leuning model, the only contribution to the variability of the ratio of WUE comes from the dependence of the CO_2 compensation point Γ on leaf temperature, and thus for the instantaneous ratio of WUE s, the only source of variability originates from the environmental forcing. Finally, concerning the transpiration reduction under eCO_2 at the hourly time scale, the variability of the reduction ratio is larger for the Ball-Berry model, while its mean value is smaller. This result seems rather insensitive to the light environment and is the same for the upper and lower levels of the canopy.

As the temporal scale increases, the variability of the responses diminishes primarily because the environmental forcing variability is reduced by the longer averaging interval. Conversely, the variability in model parameters affects all temporal scales. In other words, at the hourly scale, almost the entire variability depends on the environmental forcing, but at longer scales (e.g. the inter-annual scale), the variability of the responses depends progressively more on model parameter variability.

The leaf-level responses to eCO₂ have a pronounced seasonal and diurnal cycle (Figure 8). Carbon assimilation enhancement due to eCO₂ is larger during the summer according to both g_s conductance models. Environmental variables such as temperature and radiation are most favorable during this period (Figure 1), strengthening the effect of higher atmospheric CO₂ on photosynthesis. The effect of eCO₂ on transpiration reduction is more pronounced during winter. The composite effect of seasonality in meteorology and the reduced net CO₂ assimilation during winter result in a distinct seasonal pattern of transpiration reductions due to eCO₂.

The diurnal cycle of A_n enhancement and T reduction predicted by both g_s models is similar. The effect of eCO₂ on the transpiration reduction is less strong during midday, when A_n is larger. The only difference in terms of diurnal patterns is the late afternoon drop in A_n stimulation (and T reduction) predicted by the Leuning model. The reason for this is the sensitivity to D and the temporal lag of vapor pressure deficit D with PAR and T_a (mainly due to boundary-layer growth and heat/water vapor storage as discussed in [Matheny et al., 2014; Zhang et al., 2014]). Given that the temporal shift between RH and D to T_a and PAR is not the same, the difference between the two model predictions of A_n stimulation and T reduction can be attributed to differences in the diurnal cycles of RH and D .

3.2 Ecosystem Level

3.2.1 T&C Simulation Results

Beyond the direct effects and the role played by of environmental drivers on CO₂ and water fluxes [Holtum and Winter, 2010; De Kauwe et al., 2013], several indirect effects exist when upscaling from leaf to ecosystem level. Those effects can create compensatory mechanisms between the enhancement of net assimilation and reduction of water and carbon fluxes that complicate such upscaling [Fatichi et al., 2016b]. For instance, it is noted that eCO₂ can result in leaf area index (LAI) increases due to NPP enhancement [Kergoat, 2002; Norby et al., 2005; Dermody et al., 2007; McCarthy et al., 2007]. Increased foliage area results in larger transpiration rates that can deplete soil water, and increases canopy interception, which can further reduce water availability in the soil. However, the eCO₂ may also lead to an increased WUE , primarily due to reduced transpiration per unit leaf area that can lead to favorable soil water conditions, and thus result in an even stronger stimulation of productivity. The degree to which the effects of decreased

transpiration per unit leaf area and the increased foliage area compensated for each other is a difficult question that can be addressed by ecosystem models.

To assess how such indirect effects impact the variability of photosynthesis stimulation, transpiration reduction, *NPP* and *WUE* increase at the ecosystem level due to elevated atmospheric CO₂, the T&C model was used. The model performs reasonably well in reproducing the effects of eCO₂ on water and carbon fluxes as well as vegetation dynamics at the Duke Forest (Supplementary Material). In Figure 9, the indirect effects of eCO₂ at the ecosystem level are shown. In general, eCO₂ leads to a ~20% increase in leaf area (comparable to observed changes [McCarthy *et al.*, 2006, 2007], which in turn reduces the fraction of the foliage exposed to direct light. It also leads to higher modeled soil moisture values, although differences between the CO₂ treatments are unlikely to be large enough to modify plant water stress for most of the time. The effect of increased *WUE* on soil moisture is more pronounced during periods of drought. Finally, there is an increase in water losses due to enhanced evaporation from interception for an eCO₂ state, but those losses are low in comparison to the total precipitation at the Duke Forest. The strength of the CO₂ effect is dependent on the model parameters, with V_{cmax} being more influential than the parameters related to stomatal conductance.

The effects of the meteorological and parameter variability on the variability of carbon assimilation enhancement, transpiration reduction, and *WUE* increase are similar in magnitude and patterns to the leaf level results (Figure 10). In particular, most of the variability at the short temporal scales can be explained by the environmental drivers, whereas model parameter variability can explain most of the variability of the ecosystem responses in the longer-term. The range of temporal scales at which high-frequency meteorological variability can influence long-term variability depends on the potential of the meteorological forcing to be integrated into slow dynamics such as soil moisture dynamics [Paschalis *et al.*, 2015]. For the Duke Forest site, where water availability is rarely stressing vegetation, high-frequency meteorological forcing cannot significantly impact soil water stress, and this is the reason why there is a reduction in the contribution of meteorological variability with scale. For the pines, the magnitude of the response at both the leaf and ecosystem levels is similar. This means that either the indirect effects of LAI, soil moisture and interception are minimal, or they compensate each other. Fatichi *et al.*, [2016b] showed that the importance of these indirect effects is negatively dependent on the wetness index (annual precipitation/annual potential evapotranspiration) of the site. Given the large wetness index of the Duke FACE, the indirect effects are likely small in this location. It should be noted that in contrast to the leaf level results, at the ecosystem level, the mean values of the response at the annual scale are different from the mean value of the responses at the short (hourly and daily) scales. This effect can be explained by the

fact that the annual scale integrates the effect of the dynamically evolving leaf area, and thus the phenology of the vegetation, which is absent from the leaf level results.

Finally, there is a difference between the responses of the pines and the hardwood species in the simulations. For the hardwood species, the eCO_2 -induced reduction in transpiration is smaller and this leads to a smaller *WUE* enhancement in comparison to the pines. The reason for this behavior is two-fold. First, the reduction in transpiration is larger during winter in Duke FACE (Figure 8), when the hardwood species shed their leaves. Secondly, the hardwoods have a smaller degree of coupling between the leaf and the atmosphere due to their larger leaf sizes that increases the leaf boundary layer resistance, as shown by other studies [De Kauwe *et al.*, 2013; Medlyn *et al.*, 2015; Huang *et al.*, 2015a].

4 Discussion

The uncertainty of leaf and ecosystem level responses to eCO_2 was explored using long-term gas exchange measurements and models for leaf- and ecosystem scales. The goal was to shed light on the uncertainty of the responses across temporal scales due to variability in meteorological drivers, the uncertainty of the parameters related to leaf biochemistry and stomatal conductance, and the functional relation used to link stomatal conductance and net assimilation. Potentially, other factors can be included (e.g. soil hydraulic properties, root-density distribution, etc.) but those selected here are deemed to be common to most of the Earth System models.

The work addressed the consequences of this variability as guided by a set of questions pertinent to the development of the next generation of ecosystem models and FACE experiments: (a) Are there essential differences between various modeling parametrizations for stomatal responses to environmental factors? (b) How reliable are the observed changes in carbon assimilation enhancement, transpiration reduction and acclimation of plant to eCO_2 given such ‘internal’ ecosystem variability? (c) How can this variability be incorporated in the next generation of dynamic vegetation models?

Concerning (a), from the results of the present research, it is apparent that the selection of the model that describes the responses of stomata to environmental drivers and atmospheric CO_2 concentrations is crucial. Despite the equivalently good fit of all g_s models to the observed data (Figure 2), significant differences between the results exist depending on the model selection. It has been recently shown that the relation between stomatal conductance and net CO_2 assimilation can have strong implications for ecosystem vegetation dynamics and potentially feed-back on the global climate [Bonan *et al.*, 2014; Sato *et al.*, 2015]. Note, for instance, that all the existing models decrease stomatal conductance in response to eCO_2 regardless of the parameter values or environmental forcing (Figure 5). Measurements show that for certain

species, the g_s response to eCO_2 may be small or entirely absent [Brodribb and McAdam, 2013], even though contradictory results also exist [Franks and Britton-Harper, 2016]. Furthermore, even when accounting for the observed variability of responses as derived from observations, the value of WUE^e/WUE^a is almost prescribed in current models (Figure 7). These findings reinforce the quest for mechanistic representation of stomatal conductance [Damour et al., 2010; Medlyn et al., 2011; Fatichi et al., 2016c]. Several recent studies have attempted mechanistic description of stomatal aperture regulation [Buckley et al., 2003; Peak and Mott, 2011; Mott and Peak, 2013], which can eventually eliminate ambiguities associated with empirical (Ball-Berry and Leuning) or phenomenological (optimality) models. However, a mechanistic model of stomatal response to environmental drivers and plant water status suitable for imminent implementation in Earth System models is not available yet, despite its desirability.

Concerning (b), the results here show that a detailed characterization of leaf- and ecosystem-level responses to eCO_2 cannot be separated by temporal scale over which such responses are being evaluated. Specifically, the variability of the responses to eCO_2 at short temporal scales, commonly referred to as instantaneous, is dependent on meteorological variability. Given that meteorological variability is ‘irreducible’ in a natural system, long-term data are essential for robust quantification of ‘instantaneous’ ecosystem responses (in a statistical sense). At longer temporal scales, the primary source of variability originates from the variation of the parameters describing the photosynthetic capacity of leaves and the sensitivity of their stomatal responses to environmental drivers. Given the high leaf-to-leaf variance in such parameters even at small spatial scales, as shown in the work here, it becomes necessary to evaluate the robustness of the ecosystem responses at long (e.g. annual) time scales. At such time scales, variability in environmental drivers is reduced but variability in leaf parameters persists and introduces significant uncertainty in fluxes and stores. It should be noted that in the present study, the parameter variability ought to be limited since the study region was a uniform single-species plantation. In unmanaged ecosystems, species diversity and the uneven age of the plants would enhance the variability contribution of the “spatial” uncertainty of the parameters [e.g., Pappas et al., 2016].

The issue of spatial parameter heterogeneity and/or of subtle underlying trends in the parameters along with the large variability in boundary conditions (e.g., species diversity, soil hydraulic properties) can hamper statistical inference of response signals to eCO_2 , especially at the ecosystem scale where direct and indirect effects on plant productivity and hydrology co-exist [Holtum and Winter, 2010; Piao et al., 2013; Zaehle et al., 2014; Fatichi et al., 2016b]. For this reason, discrepancies between the results of various FACE experiments should not be surprising [Nowak et al., 2004; Körner, 2006; Norby and Zak, 2011; De Kauwe et al., 2013] and likely only a combination of data analysis from field studies and modeling may identify the causes for different responses and the real uncertainty bounds of the results.

Concerning (c), modeling procedures that incorporate both meteorological variability and the spatial variability in the photosynthetic capacity, stomatal responses to environmental variables and generally all the parameters related to plant functioning is essential. While meteorological variability is already incorporated in most modeling studies at the hourly or sub-hourly scale, the spatial variability of the parameters related to plant functioning is more difficult to address. The emergence of trait-based approaches in vegetation modeling can be regarded as a reasonable solution [Scheiter *et al.*, 2013; Bodegom *et al.*, 2014; Pappas *et al.*, 2016]. Such an approach can inherently incorporate the variability and stochasticity of the plant traits within and between ecosystems. Using such approaches to quantify variability of the responses of ecosystems is also straightforward, since the deterministic framework of PFTs is loosened and diversity/variability of plants traits can be explicitly taken into account with stochastic analyses. This approach has only been recently incorporated into large-scale ecosystem models but it is promising to address uncertainty of spatially and potentially temporally variable parameters [Pavlick *et al.*, 2013; Scheiter *et al.*, 2013; Sakschewski *et al.*, 2015].

5 Conclusions

The leaf and ecosystem level variability of the responses of carbon gain, transpiration and water use efficiency to elevated CO₂ was considered for a wide range of temporal scales at the Duke FACE experiment. Using an extensive data set consisting of more than 500 $A_n - c_i$ curves collected over a 10 year period, we estimated the variability of the parameters related to the photosynthetic machinery of the leaves and the response of their stomata to environmental drivers, and the impacts of carbon and nitrogen fertilization on those parameters. Combining the results of the data analysis with modeling approaches at the leaf (three stomatal conductance models; Ball-Berry, Leuning and optimality model) and the ecosystem scale model T&C, the variability of the responses due to meteorological drivers and model parameter uncertainty was partitioned and analyzed.

The key findings are:

- (a) The variability of the parameters describing photosynthetic capacity and the responses of stomata to environmental drivers and CO₂ concentrations is large, complicating the identification of transient patterns, such as acclimation to eCO₂ or nutrient feedbacks, and suggesting that extensive data collection is essential for such identification, even in a uniform plantation.
- (b) While the three commonly used stomatal conductance models describe the gas-exchange measurements reasonably for ambient and enriched atmospheric CO₂, there are fundamental differences between their responses to environmental drivers and their interpretation of the

marginal water use efficiency. Those differences may lead to important long-term consequences in simulated plant water stress and thus vegetation growth and mortality.

(c) The variability in the responses to elevated CO₂ of photosynthesis stimulation, transpiration reduction and water use efficiency enhancement depends on the scale at which the responses are being evaluated. Variability in leaf-level fluxes at short temporal scales can be mainly attributed to meteorological drivers. As temporal scales increase, the contribution of the uncertainty in the model parameters becomes predominant.

(d) The ecosystem-level responses to elevated CO₂ were comparable to those at the leaf level at the Duke FACE. Indirect effects related to the impact of leaf-level photosynthesis stimulation and stomatal conductance reduction, such as LAI increase and more favorable soil water conditions exist, however, compensatory effects tended to weaken these indirect effects, producing an overall response similar to the leaf-level response.

Quantification of the effects of increased atmospheric CO₂ concentrations are essential for understanding the coupled bidirectional effects of anthropogenic carbon emissions on global vegetation and climate. As both stomatal regulation in response to environmental drivers and internal ecosystem variability play major roles, the evidence presented in this study may spur research for mechanistic models of stomatal functioning, which will be essential for the next generation of terrestrial ecosystem models.

Acknowledgements

The authors are grateful to all individuals of the FLUXNET project (<http://fluxnet.ornl.gov/>) and the researches of the Duke Forest involved in the collection of the data used in this study. AP acknowledges the financial support of the Swiss National Sciences Foundation (grant No P2EZP2_152244) and the Stavros Niarchos Foundation, through the SNSF Early Postdoc Mobility Fellowship. SF thanks the support of the Stavros Niarchos Foundation and the ETH Zurich Foundation (Grant ETH-29 14-2). GK and SP acknowledge support from the U.S. National Science Foundation (NSF-EAR-1344703), the U.S. Department of Energy (DOE) through the office of Biological and Environmental Research (BER) Terrestrial Ecosystem Science (TES) Program (DE-SC0006967, DE-SC0011461, DE-SC0006700). DW acknowledges support from a Canadian Natural Sciences and Engineering Research Council Discovery grant and an Ontario Early Researcher Award.

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List of Tables

Table 1: Number of $A - c_i$ curves included at the data analysis

	Ambient CO ₂				Elevated CO ₂			
	Upper		Lower		Upper		Lower	
	New	Old	New	Old	New	Old	New	Old
no N Fertilization	53	79	45	37	33	64	26	34
N Fertilization	31	9	29	7	10	9	9	9

Table 2: p-values of the 4-way ANOVA for V_{cmax} , J_{max} , m_b , m_l , λ , according to the CO₂ fertilization (C), Nitrogen fertilization (N), Canopy level (L) and Age class (A). Values below 5%, where the difference of the mean values of each of the dependent variables (V_{cmax} , J_{max} , m_b , m_l , λ) are statistically significant based on the discrete dependent variables (C, N, L, A) marked as bold. Covariation between the dependent variables is neglected.

	V_{cmax}	J_{max}	m_b	m_l	λ
C	0.8198	0.8632	<0.0001	<0.0001	0.2187
N	0.9713	0.0348	<0.0001	<0.0001	<0.0001
L	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
C*N	0.2814	0.2215	<0.0001	<0.0001	0.0283
C*L	0.6183	0.5745	<0.0001	0.0003	0.2553
C*A	0.8797	0.9018	<0.0001	<0.0001	<0.0001
N*L	0.2490	0.5534	<0.0001	<0.0001	0.0057
N*A	0.1084	<0.0001	<0.0001	<0.0001	0.4524
L*A	0.0011	0.1440	<0.0001	<0.0001	0.2224
C*N*L	0.4530	0.9175	<0.0001	<0.0001	0.8188
C*N*A	0.0972	0.9791	0.0022	<0.0001	0.3420
C*L*A	0.8968	0.5492	<0.0001	<0.0001	0.0565
N*L*A	0.1342	0.8288	<0.0001	<0.0001	0.2688
C*N*L*A	0.6623	0.3119	<0.0001	0.4495	0.8237

Table 3: Standard deviations of the simulated ratios of net photosynthesis (A_n), water use efficiency (WUE) and transpiration (T) for two canopy levels (upper sun leaves, lower shaded leaves), three time scales (1 hour, 1 day, 1 year) according to the Ball-Berry and the Leuning stomatal conductance model. σ_{TOT} , σ_{PAR} , σ_{ENV} represent the standard deviations of the sets S_{TOT} , S_{PAR} , S_{ENV} respectively. The three sets lump the variability due to CO_2 and N treatments and leaf age. The same data used in this table are used for the illustration of the variability of the responses in Figure 7.

	A_n^e/A_n^a			WUE^e/WUE^a			T^e/T^a		
	σ_{TOT}	σ_{PAR}	σ_{ENV}	σ_{TOT}	σ_{PAR}	σ_{ENV}	σ_{TOT}	σ_{PAR}	σ_{ENV}
<i>Sun Leaves</i>									
<i>Ball-Berry</i>									
1 hour	0.240	0.042	0.236	0.000	0.000	0.000	0.162	0.036	0.158
1 day	0.166	0.037	0.162	0.036	0.010	0.034	0.182	0.032	0.179
1 year	0.039	0.035	0.018	0.022	0.018	0.013	0.040	0.036	0.018
<i>Leuning</i>									
1 hour	0.149	0.020	0.148	0.025	0.000	0.025	0.086	0.016	0.084
1 day	0.113	0.018	0.112	0.017	0.006	0.016	0.074	0.014	0.073
1 year	0.022	0.019	0.011	0.014	0.012	0.006	0.021	0.018	0.009
<i>Shaded Leaves</i>									
<i>Ball-Berry</i>									
1 hour	0.171	0.038	0.168	0.000	0.000	0.000	0.117	0.034	0.113
1 day	0.594	0.290	0.559	0.200	0.065	0.190	0.872	0.502	0.791
1 year	0.052	0.049	0.018	0.055	0.023	0.050	0.053	0.050	0.020
<i>Leuning</i>									
1 hour	0.112	0.018	0.110	0.025	0.000	0.025	0.063	0.015	0.062
1 day	0.096	0.019	0.094	0.025	0.003	0.025	0.074	0.013	0.072
1 year	0.018	0.015	0.009	0.010	0.008	0.006	0.016	0.014	0.009

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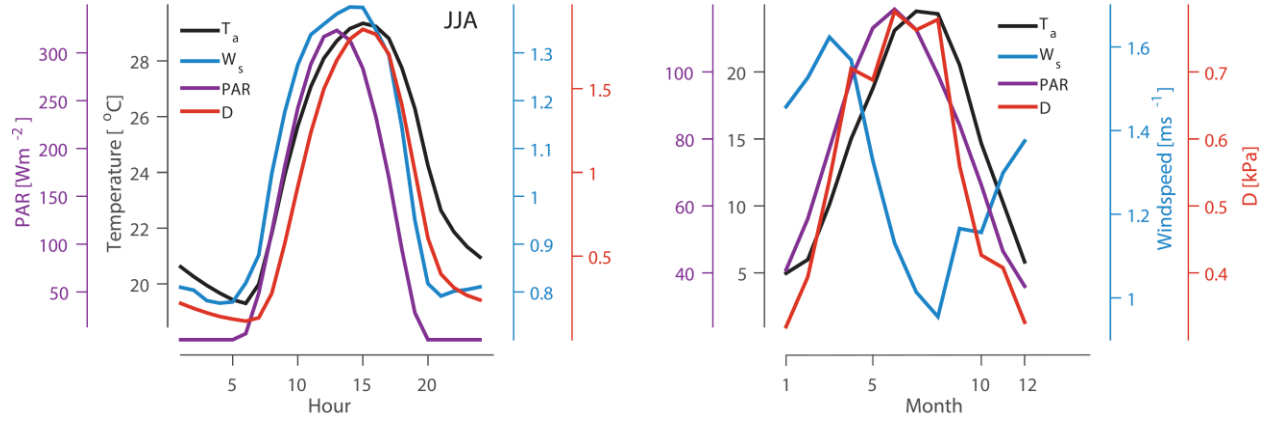


Figure 1: Meteorological forcing observed at the Duke Forest. In the left panel the diurnal cycle air temperature (T_a), windspeed (W_s), photosynthetic active radiation (PAR) and vapor pressure deficit (D) for summer (June-July-August), and in the left panel the seasonal cycle of the same variables is shown. Axes colors (black, blue purple and red) correspond to the color lines of the respective variables (T_a , W_s , PAR, D).

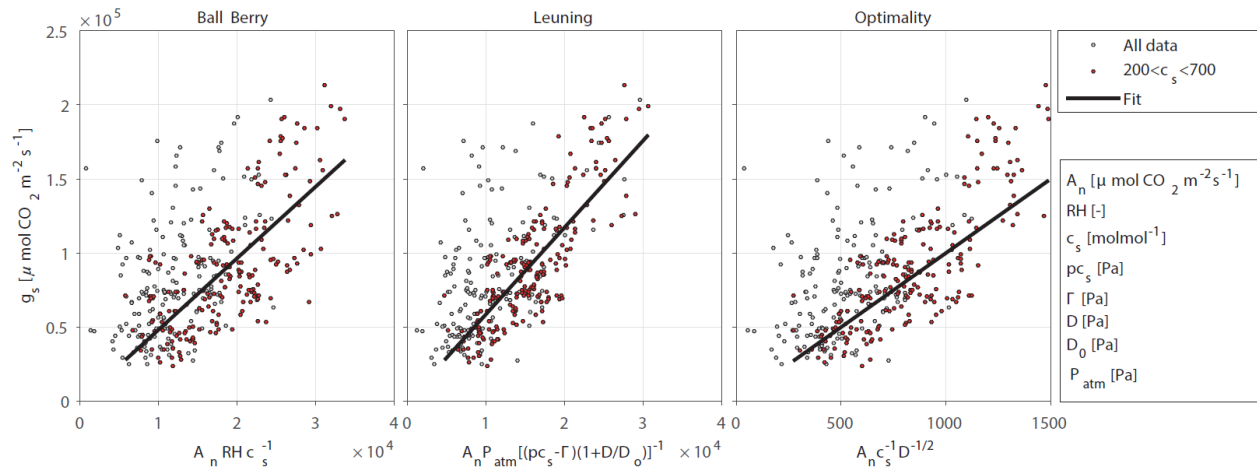


Figure 2: Typical agreement between measured and modeled stomatal conductance (g_s) for the three stomatal conductance models. Grey points represent the entire data set with no filtering, whereas red dots represent only the data points for which the CO₂ concentration at the leaf surface (c_s) was in the range: $200 \text{ ppm} < c_s < 700 \text{ ppm}$.

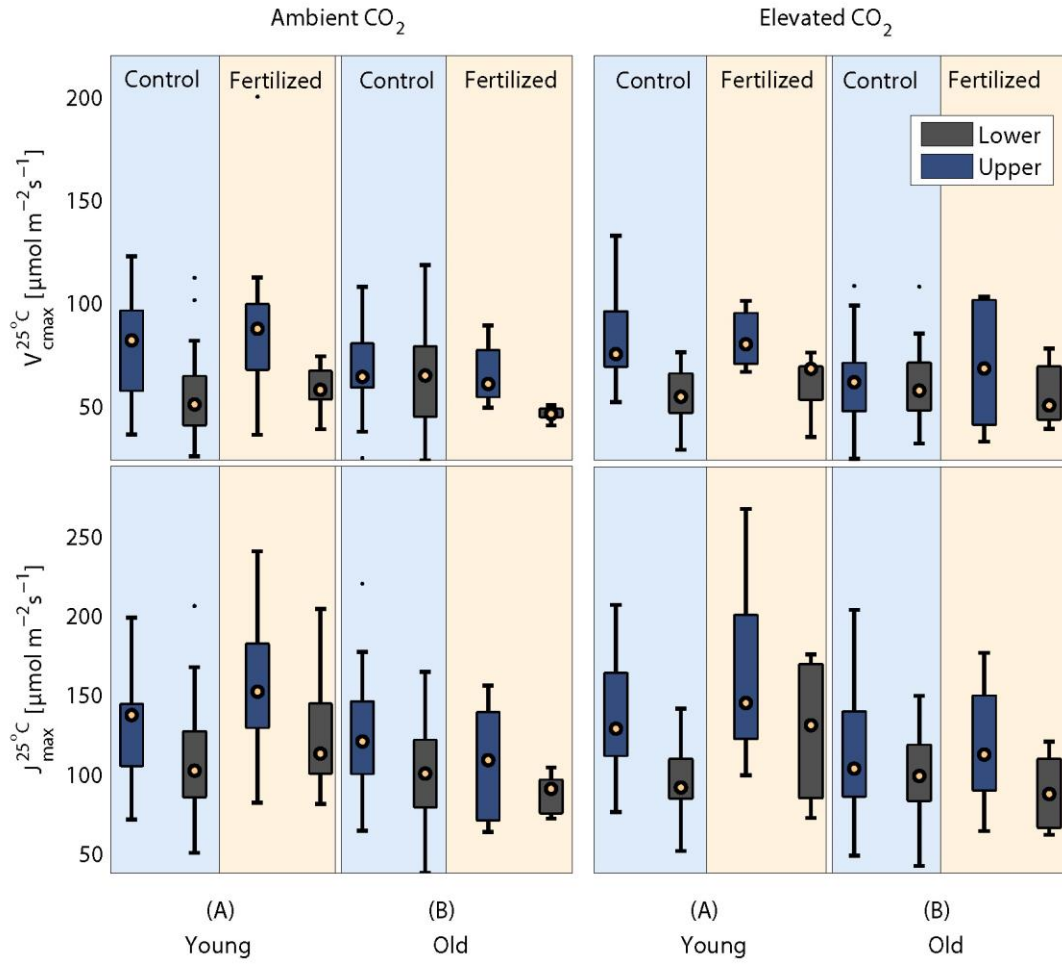


Figure 3: Boxplots showing the variability of V_{max} (upper panels) and J_{max} (lower panels) at 25°C for every class of CO_2 , N fertilization, leaf age and canopy level. Boxes refer to the 25%-75% percentiles and whiskers to the 5%-95% percentiles. The left panels represent plants grown under ambient CO_2 , and the right panels plants grown under elevated CO_2 . Panels marked as (A) represent new needles and (B) old needles. Blue background color represents unfertilized and yellow background color represents N-fertilized trees. Blue boxplots represent measurement taken at the upper part of the canopy and grey boxplot measurements at the lower part.

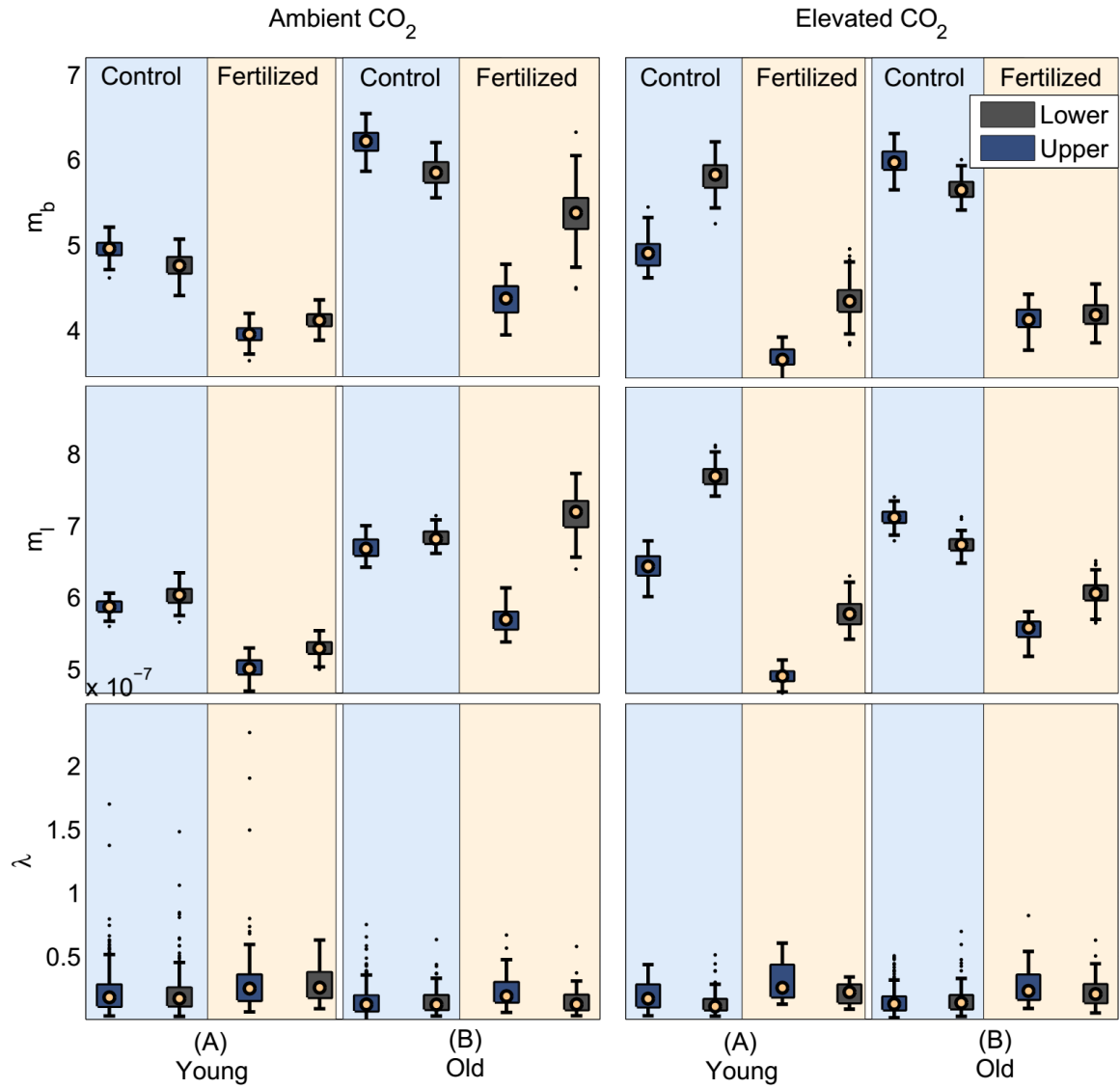


Figure 4: Same as Figure 3, but for the parameters related to the stomatal conductance models. m_b is the parameter of the Ball-Berry stomatal conductance model, m_l is the parameter related to the Leuning model and λ is the marginal water use efficiency used in the optimality model.

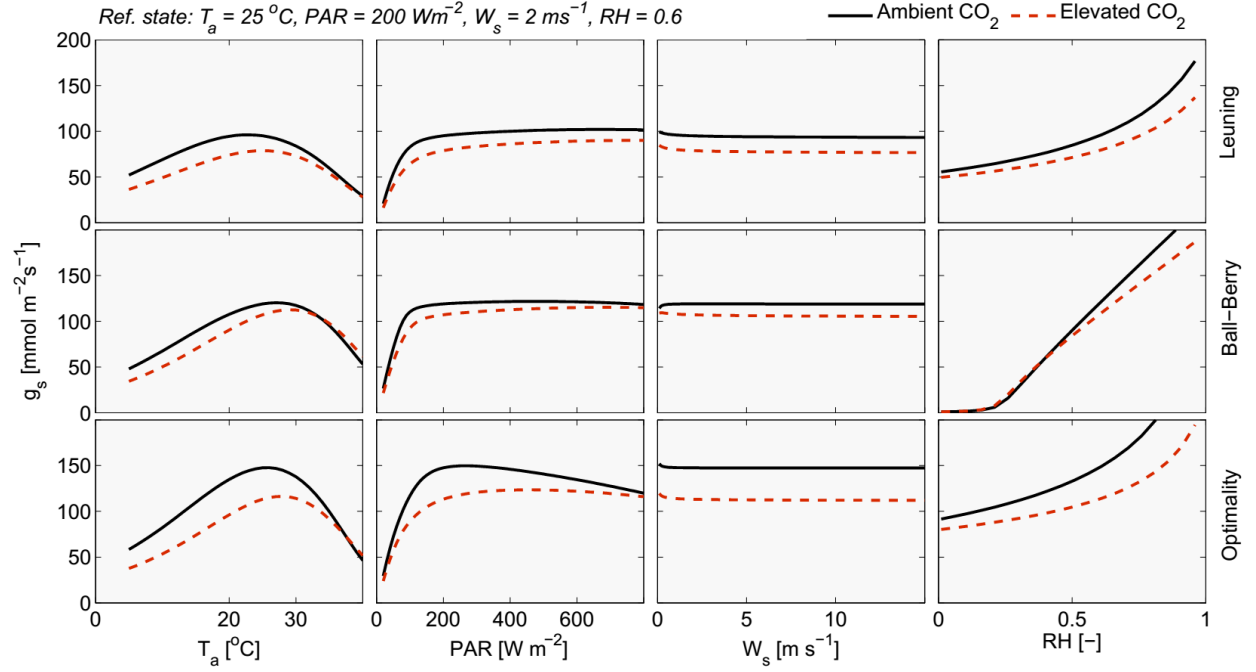


Figure 5: Modeled stomatal conductance according to the Leuning (upper panels), Ball-Berry (middle panels) and the optimality model (lower panels). For each subplot all meteorological variables were held constant and equal to the reference state ($T_a=25^\circ\text{C}$, $PAR = 200 \text{ W m}^{-2}$ ($\sim 915 \mu\text{mol m}^{-2} \text{ s}^{-1}$), $W_s = 2 \text{ m s}^{-1}$, $RH = 0.6$ [-]), except the variable of interest featured on the corresponding x-axis. The parameters used are: $V_{cmax} = 80 \mu\text{mol m}^{-2} \text{ s}^{-1}$, $J_{max} = 160 \mu\text{mol m}^{-2} \text{ s}^{-1}$, $m_l = 6$, $m_b = 5$, $\lambda = 10^{-8} [\text{mol mol}^{-1} \text{ Pa}^{-1}]$

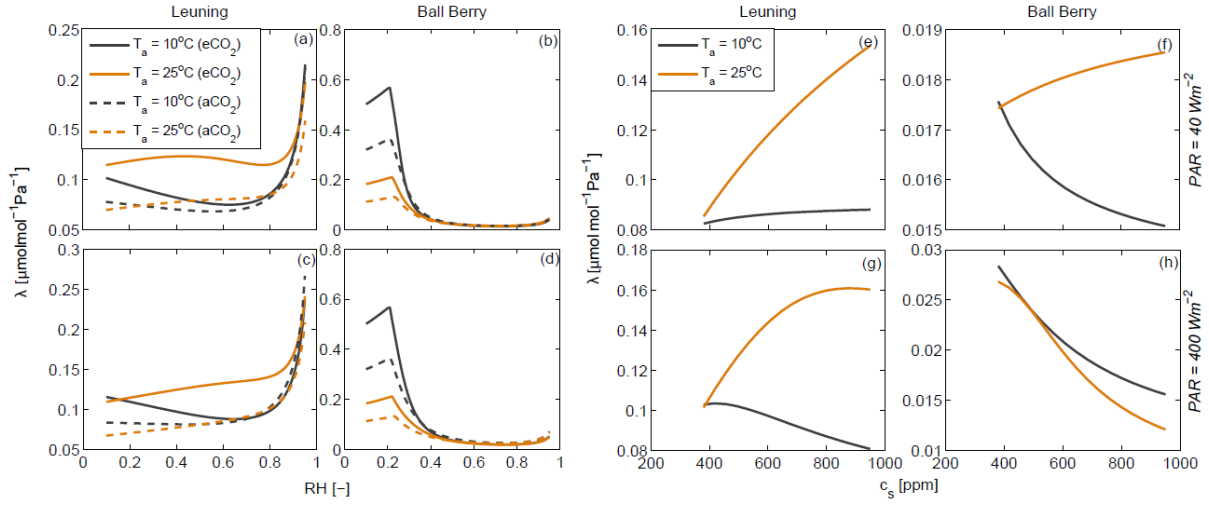


Figure 6: Marginal water use efficiency λ [$\mu\text{molCO}_2 \text{ molH}_2\text{O}^{-1} \text{ Pa}^{-1}$] (for this calculation the unit convention follow [Katul *et al.*, 2010]) as predicted by the Leuning and the Ball-Berry models for several combinations of meteorological forcing (left panels) and CO₂ concentrations (right panels).

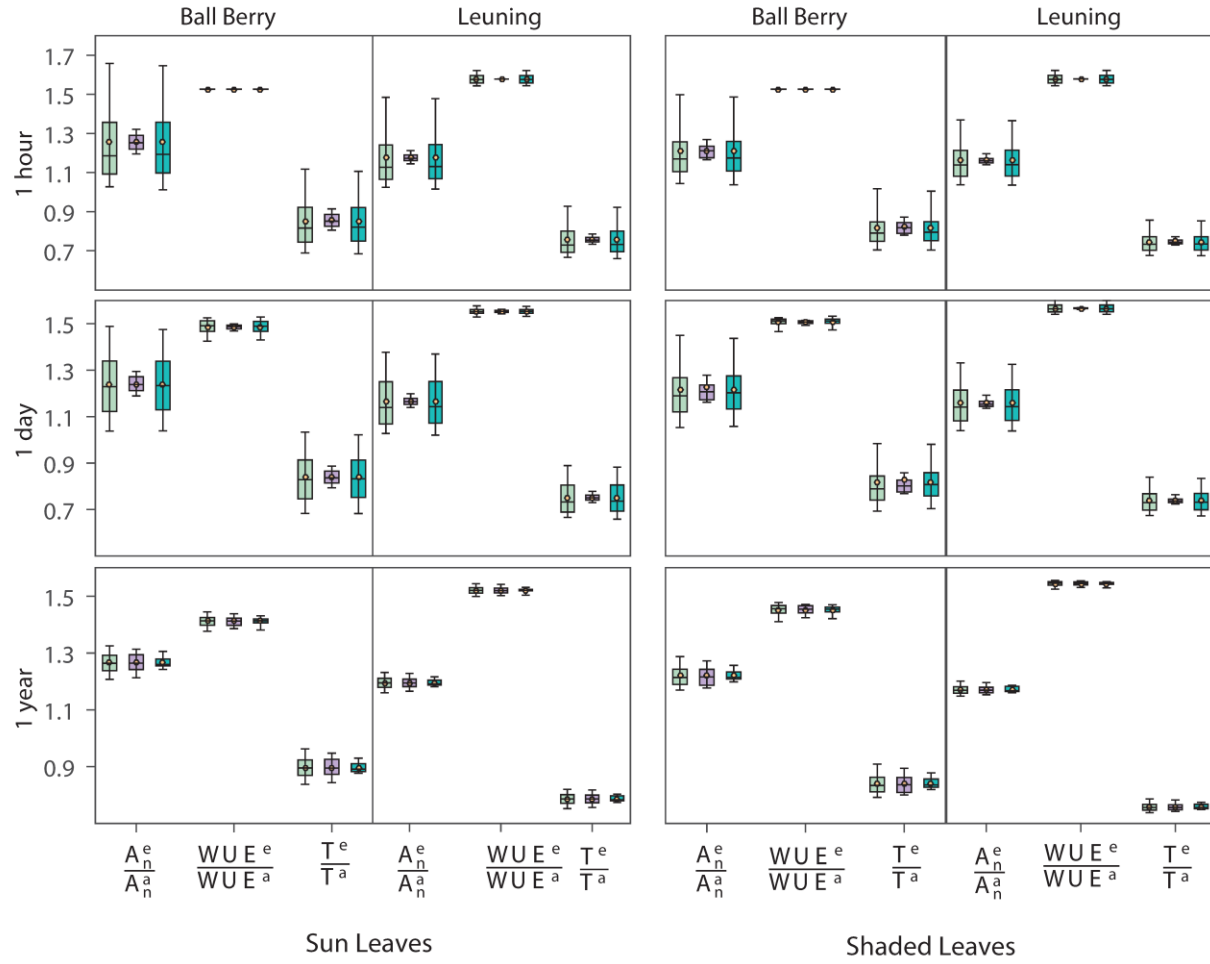


Figure 7: Boxplots representing the total variability (left green boxplots), variability due to model parameters only (middle purple boxplots) and variability due to meteorological forcing only (right blue boxplots) of the ratios $\frac{A_n^e}{A_n^a}$, $\frac{WUE^e}{WUE^a}$, $\frac{T^e}{T^a}$ estimated based on the Ball-Berry and the Leuning models for A_n , WUE , and T due to eCO_2 integrated at the time scales of 1 hour (upper panels), 1 day (middle panels) and 1 year (lower panels) for leaves exposed to full light (left 2 panels) and shaded leaves (right 2 panels).

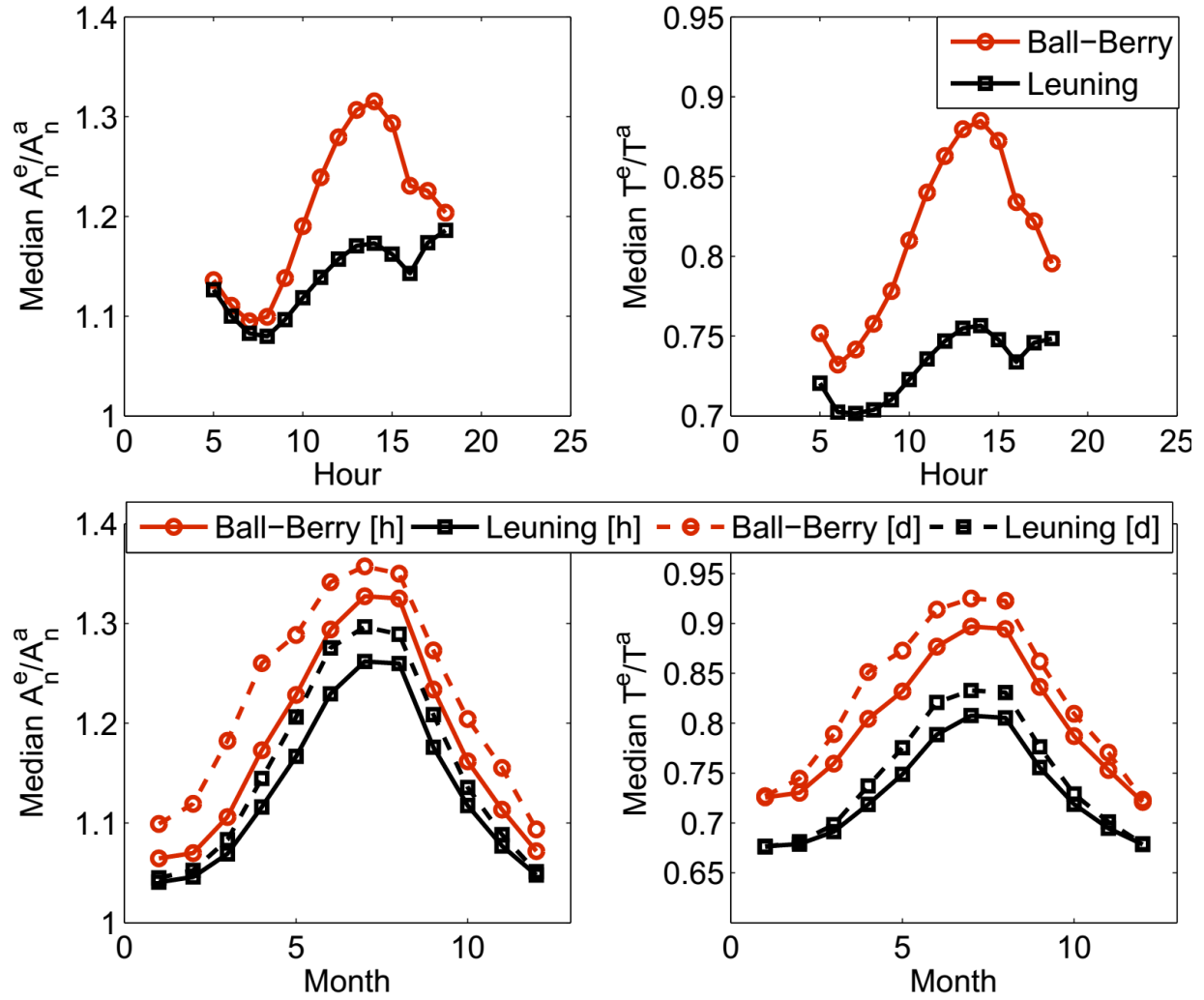


Figure 8: Median values of the diurnal (upper panels) and seasonal (lower panels) cycles of the net assimilation stimulation (left) and transpiration reduction (right) modeled using the Ball Berry (red lines) and the Leuning (black lines) model, for the hourly (continuous lines) and daily (dashed lines) time scale.

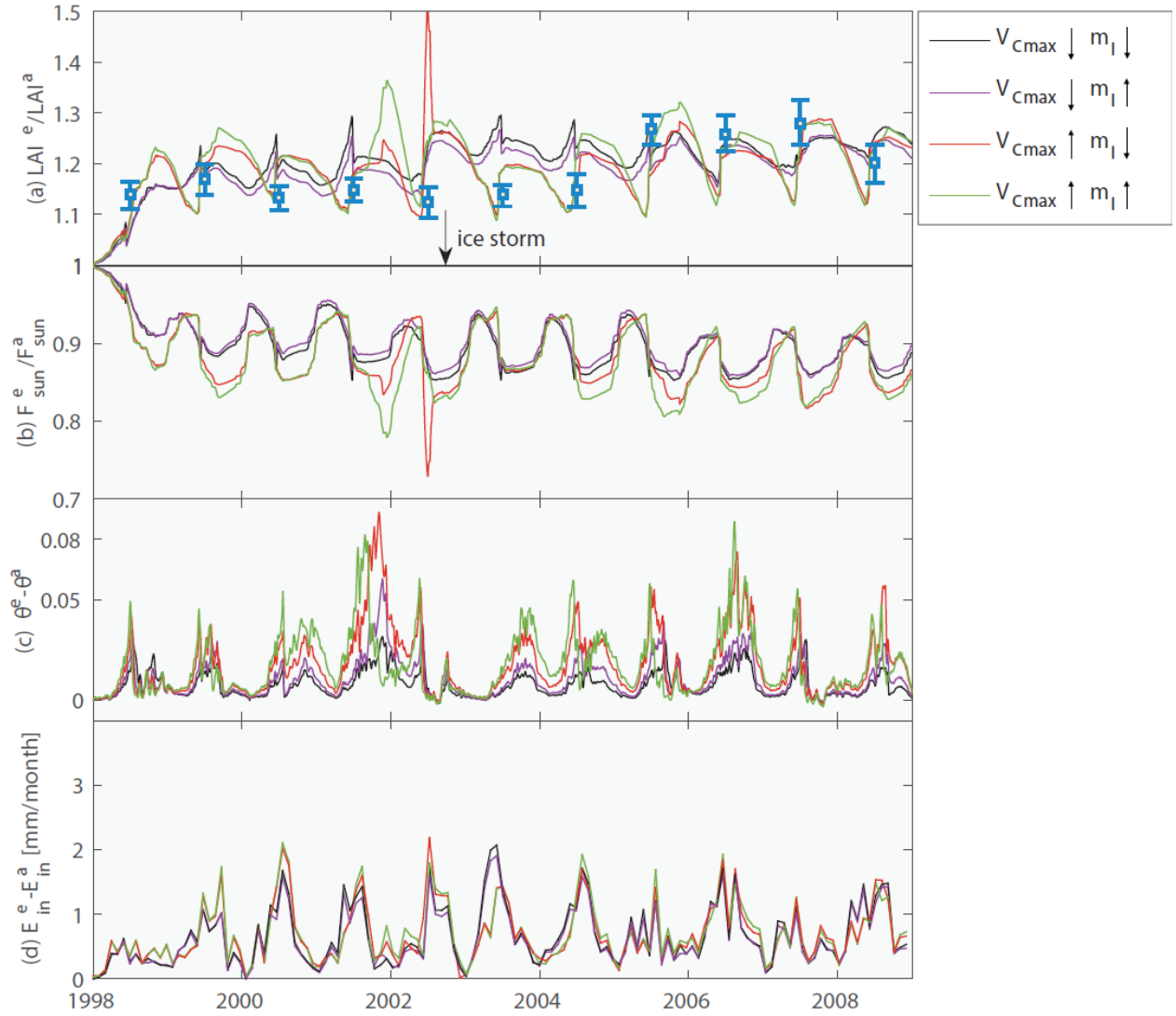


Figure 9: Modeled temporal evolution of the eCO₂ effects: (a) LAI enhancement, (b) reduction of the fraction of canopy area exposed to sun, (c) soil moisture enhancement (d) increase in evaporation losses from interception for the overstory pines. The various combinations of V_{cmax} and m_l are shown in the legend. Arrows pointing up relate to high values and arrows pointing down to low values of the respective parameter. The blue errorbars in (a) represent the time evolution of the observed LAI enhancement. The range of the errorbars represent the standard deviation of the measured LAI enhancement within a year. The arrow in (a) represents the occurrence of an ice storm that caused damages to the vegetation.

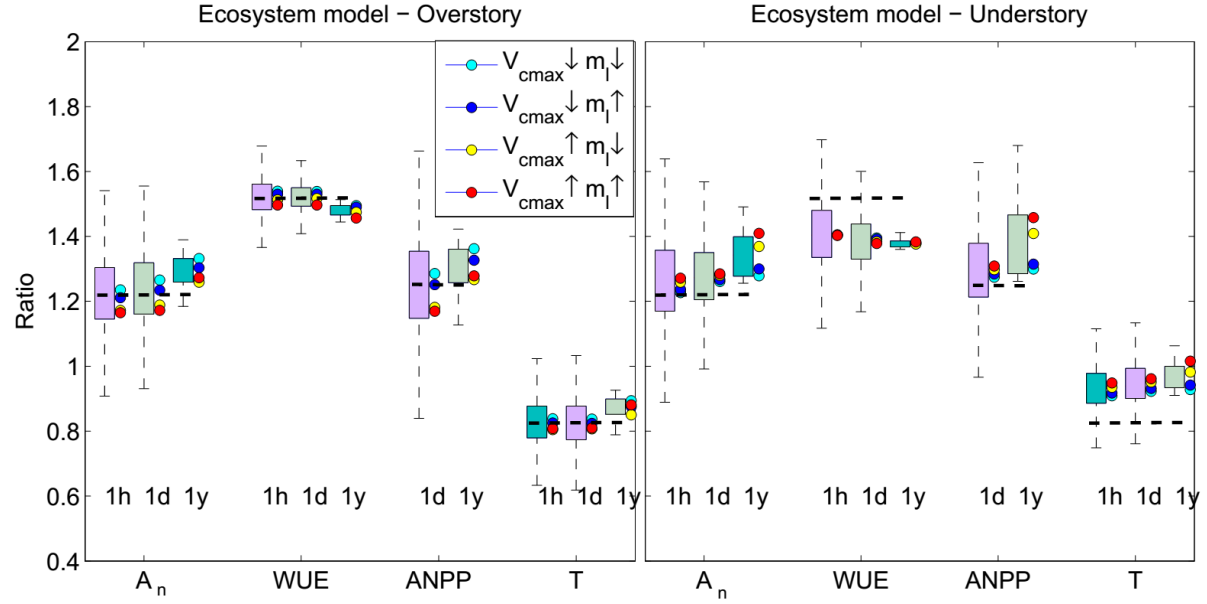


Figure 10: Ecosystem level variability of the responses of A_n , WUE , aboveground NPP and T for the overstory pines (left panel) and the understory hardwood species (right panel) as simulated by the T&C model. The boxplot represent the total variability for 3 different time scales (1hour, 1day and 1 year) and the dots the mean value of the ecosystem response according to the combination of the V_{cmax} and m_l parameters. The dashed lines represent the average response of the pines.