On the complementary relationship between marginal nitrogen and water-use efficiencies among Pinus taeda leaves grown under ambient and CO₂-enriched environments

Sari Palmroth1,2,*, Gabriel G. Katul1,3, Chris A. Maier4, Eric Ward1, Stefano Manzoni1,3 and Giulia Vico3,5

1Nicholas School of the Environment, Box 90328, Duke University, Durham, NC 27708, USA, 2Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden, 3Civil and Environmental Engineering Department, Box 90287, Duke University, Durham, NC 27708-0287, USA, 4Southern Research Station, USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC 27709, USA and 5Department of Crop Production Ecology, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

* For correspondence. E-mail sari.palmroth@duke.edu

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- **Background and Aims** Water and nitrogen (N) are two limiting resources for biomass production of terrestrial vegetation. Water losses in transpiration (E) can be decreased by reducing leaf stomatal conductance (gs) at the expense of lowering CO₂ uptake (A), resulting in increased water-use efficiency. However, with more N available, higher allocation of N to photosynthetic proteins improves A so that N-use efficiency is reduced when gs declines. Hence, a trade-off is expected between these two resource-use efficiencies. In this study it is hypothesized that when foliar concentration (N) varies on time scales much longer than gs, an explicit complementary relationship between the marginal water- and N-use efficiency emerges. Furthermore, a shift in this relationship is anticipated with increasing atmospheric CO₂ concentration (cₐ).

- **Methods** Optimization theory is employed to quantify interactions between resource-use efficiencies under elevated cₐ and soil N amendments. The analyses are based on marginal water- and N-use efficiencies, \( \lambda = (\delta A/\delta g_s)/ (\delta E/\delta g_s) \) and \( \eta = \delta A/\delta N \), respectively. The relationship between the two efficiencies and related variation in intercellular CO₂ concentration (cₐ) were examined using A/E and foliar N measured on Pinus taeda needles collected at various canopy locations at the Duke Forest Free Air CO₂ Enrichment experiment (North Carolina, USA).

- **Key Results** Optimality theory allowed the definition of a novel, explicit relationship between two intrinsic leaf-scale properties where \( \lambda \) is complementary to the square-root of \( \eta \). The data support the model predictions that elevated cₐ increased \( \eta \) and \( \lambda \), and at given cₐ and needle age-class, the two quantities varied among needles in an approximately complementary manner.

- **Conclusions** The derived analytical expressions can be employed in scaling-up carbon, water and N fluxes from leave to ecosystem, but also to derive transpiration estimates from those of \( \eta \), and assist in predicting how increasing \( c_a \) influences ecosystem water use.

**Key words:** Elevated CO₂, FACE, fertilization, leaf gas exchange, nitrogen, optimal stomatal conductance, pine, Pinus taeda, water-use efficiency.

INTRODUCTION

Ecosystem productivity and carbon storage in plant biomass are essential components of global carbon balance but their estimates remain highly uncertain. Both processes are controlled by soil nitrogen (N) availability, which determines the rate of production of different biomass compartments (McMurtrie and Wolf, 1983). In turn, foliar N concentration (N) influences leaf photosynthetic capacity (Field and Mooney, 1986; Evans, 1989). Because soil N availability is often limited and acquiring it has a cost (Bloom et al., 1992), N and carbon are invested in upper canopy foliage where the return (in terms of CO₂ uptake) is larger because light is not limiting photosynthesis (e.g. Field, 1983; Anten et al., 1995; Kull and Krujút, 1999; Dewar et al., 2012; Peltoniemi et al., 2012). This pattern explains most of the variability of within-canopy N per unit leaf area (Nₐ) at a given atmospheric CO₂ concentration (cₐ).

Actual leaf CO₂ uptake depends not only on leaf biochemistry (demand for CO₂) but also on the diffusion rate of CO₂ from the atmosphere through stomata to the carboxylation sites (CO₂ supply). The diffusion rate reflects the concentration gradient driving CO₂ uptake and the degree of stomatal opening, which impacts stomatal conductance (gs). The CO₂ gradient is generally enhanced by elevated cₐ and increased N allocation to photosynthetic enzymes (that reduce the concentrations of CO₂ at carboxylation sites and hence in the leaf air space, cₐ). However, elevated cₐ decreases stomatal opening in some species (Medlyn et al., 2001), and reduction of gs limits CO₂ uptake and reduces transpirational water loss at the scale of the leaf. In addition, under elevated cₐ, the relationship between photosynthetic capacity of leaves and N may shift due to acclimation of photosynthetic biochemistry, resulting in smaller N investment in carboxylation-related proteins (reviewed by Ainsworth and...
Carbon and water exchange of leaves in response to varying CO₂ and N supply and methods of up-scaling are used in ecosystem carbon–water–nitrogen models, including large-scale climate models to assess the effects of elevated atmospheric CO₂ and N deposition on regional carbon fluxes and atmospheric CO₂ concentrations (Bonan, 2008). The incorporation of leaf-level functions to large-scale models has been made possible through remotely sensed estimates of canopy N, mapped over regions and continents (Ollinger et al., 2008), and within-canopy radiative transfer and resource (C and N) allocation schemes. Stomatal conductance of leaves, based on the optimality hypothesis, offers an alternative for the current semi-empirical formulations in ecosystem models (Launainen et al., 2011; Manzoni et al., 2011a). Moreover, such an up-scaling scheme can also be employed to study how variations in intrinsic variables of leaves, such as marginal water and N-use efficiencies, are reflected in ‘effective’ canopy (or big-leaf) properties and gas exchange by ecosystems. These ‘canopy-level’ functions are likely to be more easily incorporated in or used to constrain large-scale models.

In this work, we hypothesize that when the timescale of variation of gc is much shorter than those of N variations for leaves operating at optimal gc, there will be an explicit relationship between the marginal water and N-use efficiency. This expression differs from previous trade-off hypotheses between N and water use because (a) it is based on a complementary relationship among intrinsic variables (η and λ), and (b) this relationship is a consequence of optimal stomatal regulation on short time scales and the difference in timescale between N and water use. It has been shown previously that, at a given stomatal conductance and N, when photosynthesis is primarily limited by the amount and activity of Rubisco, increasing c₂ increases λ (Katul et al., 2010; Manzoni et al., 2011b). Increasing c₂ increases c₁ and shifts their relationship so that λ and η both increase. To test this complementarity hypothesis, and to study how the relationship may be affected by soil N additions, we quantified the variability of N, and water-use efficiencies among leaves using gas-exchange measurements (A–C_i curves) collected at different times and canopy positions in the Pinus taeda stand of the Duke Forest Free Air CO₂ Enrichment (FACE) experiment. At Duke FACE trees were grown under a split-pilot design of elevated atmospheric CO₂ (+200 μmol mol⁻¹) and soil N amendments.

Our analysis focuses on time scales commensurate with the averaging times typical of gas-exchange measurements (i.e. hours). This is different from assessing how these resources are used by an individual plant over longer time scales (e.g. biomass growth), which requires longer integration times and accounting for changes in biomass and its partitioning (Dewar et al., 2009). We use the optimality model and data simultaneously as a diagnostic tool to interpret the measured leaf gas exchange, assuming leaves are operating within the confines of optimality theory. Previous gas-exchange studies on P. taeda and P. sylvestris trees suggest that leaves tend to operate near their optimal stomatal conductance irrespective of climatic conditions and c₂ (Palmroth et al., 1999; Katul et al., 2010). Here, we further assume that the measured gas-exchange rates reflect growth conditions of the needles, and assess the effects of elevated c₂ and N availabilities on the derived relationship between η and λ.
MATERIALS AND METHODS

Theory

CO₂ uptake–stomatal conductance relationship. Mass transfer of CO₂ and water vapour through leaves occurs via Fickian diffusion effectively described as

\[ A = g_c(c_a - c_i) \]  

(1)

\[ E = g_w(e_i - e_a) \approx g_w D \]  

(2)

where \( c_a \) and \( c_i \) are the ambient and intercellular CO₂ concentrations, respectively, \( g_c \) is the stomatal conductance to CO₂, \( g_w \) is conductance to water vapour, \( e_i \) and \( e_a \) are the intercellular and ambient water-vapour concentrations, respectively, and \( D \) is the vapour-pressure deficit approximating \( e_i - e_a \). Because of the difference in relative diffusivity of water and CO₂, vapour and CO₂, respectively, and because of the difference in relative diffusivity of water vapour and CO₂, \( g_w \approx 1 \) for \( g_c \). Boundary-layer conductance is assumed to be much larger than stomatal conductance, which is typical in the cuvette-based gas-exchange measurements used in this study. Hence, leaf temperature can be well approximated by air temperature.

Equation (1) describes the rate of CO₂ supplied from the atmosphere to the leaf at a given \( c_i \), where \( c_i \) depends on the balance between this atmospheric CO₂ supply and demand by the photosynthetic biochemistry. The CO₂ demand can be generically expressed as (Farquhar et al., 1980)

\[ A = \frac{a_1(c_i - c_r)}{c_1 + a_2} - R_d \]  

(3)

where \( I^w \) is the CO₂ compensation point in the absence of mitochondrial respiration, \( a_1 \) and \( a_2 \) are kinetic constants that depend on whether photosynthesis is limited by ribulose-1,5 biphosphate (RuBP) regeneration rate or Rubisco activity, and \( R_d \) is the daytime photosynthetic respiration rate. Under light-saturated conditions, as in all gas-exchange measurements used in this study, \( a_1 = V_{c,max} \) (maximum carboxylation rate of RuBP) and the half-saturation constant is \( a_2 = K_c(1 + C_O/K_O) \) (where \( K_c \) and \( K_O \) are the Michaelis–Menten constants for CO₂ fixation and oxygen inhibition, respectively, and \( C_O \) is the oxygen concentration in air). These expressions for \( a_1 \) and \( a_2 \) are valid when mesophyll conductance \( (g_m) \) is non-limiting. Should \( g_m \) become important, the value of \( a_1 \) can be interpreted as a 'macroscopic' kinetic constant that also accounts for any leaf internal diffusive limitations.

Whenever \( I^w \ll c_i \), the demand function (eqn 3) may be simplified by noting that \( c_i \) in the denominator can be broken down into a long-term mean value, i.e. \( c_i = r c_a \), where \( r \) is a constant and its fluctuations assumed to be much smaller than \( a_2 \). This assumption is reasonable for light-saturated conditions given that \( a_2 \gg r \). Expressed in terms of \( g_c \) and upon neglecting \( R_d \) relative to \( A \) (as is the case for large \( A \)), eqn (1) and the linearized demand function can be combined to yield an \( A-g_c \) relationship independent of \( c_i \) (see Lloyd, 1991; Katul et al., 2010)

\[ A = \frac{g_c a_1 c_a}{a_1 + g_c (a_2 + r c_a)} \]  

(4)

It is noted that eqn (4) retains the non-linear relationship between \( A \) and \( c_a \) despite the linearized \( A-c_i \).

Marginal water-use efficiency. The theoretical optimal \( g_c \) is derived from the maximization of the objective function

\[ f(g_c) = A(g_c) - \lambda E(g_c), \]  

where \( \lambda = \frac{\partial A/\partial g_c}{\partial E/\partial g_c} \) is the marginal water-use efficiency (Hari et al., 1986; Lloyd, 1991). By inserting eqns (2) and (4) into \( f(g_c) \), a \( \lambda_{LI} \) (where LI refers to the linearized demand function) can be computed for \( \partial f(g_c)/\partial g_c = 0 \) as

\[ \lambda_{LI} = \frac{c_a}{aD} \left( 1 - \frac{c_i}{c_a} \right)^2 \]  

(5)

Moreover, the flux-based WUE may be expressed as a function of \( \lambda_{LI} \) as

\[ \text{WUE} = \frac{A}{E} = \sqrt{\frac{\lambda_{LI} c_a}{aD}} \]  

(6)

A number of studies have shown that \( \lambda_{LI} \) increases almost linearly with \( c_a \) [i.e. \( \lambda_{LI} = \lambda_0 \frac{c_a}{c_i} \)], where \( \lambda_0 \) reflects the marginal water-use efficiency of the leaf grown at \( c_i \) and results in a quasi-linear increase of WUE with \( c_a \) at a given \( D \) (Buckley, 2008; Katul et al., 2009, 2010; Barton et al., 2012; Manzoni et al., 2011b). Note, however, that the sensitivity of \( \lambda_{LI} \) to \( c_a \) in the solution of the optimal conductance depends on the assumed limiting condition of photosynthesis in the objective function. In line with the gas-exchange measurements carried out in this study, our formulation of optimal \( g_c \) (Katul et al., 2010) uses the Rubisco-limited function of the biochemical model (Farquhar et al., 1980) and results in \( \lambda_{LI} \) increasing with \( c_a \). This differs from the formulation by Medlyn et al. (2011), where photosynthesis is assumed to be limited by RuBP regeneration, so that \( \lambda_{LI} \) (or their ‘water cost of carbon’, corresponding to \( 1/\lambda_{LI} \) using our notation) is insensitive to \( c_a \). Despite the apparent contrast in the predictions of changes in marginal water-use efficiency with atmospheric \( c_a \) between these two approximations of optimal stomatal conductance, both recover the linear relationship between \( g_c \) and \( A/c_a \) used in semi-empirical models (Laumänien et al., 2011; Volpe et al., 2011; Way et al., 2011) and both suggest that stomatal conductance of P. taeda is insensitive to \( c_a \).

Marginal N-use efficiency. The derivation of \( \lambda \) can be modified to include the simultaneous costs of using water (at a given rate of \( E \)) and \( N \) (Buckley et al., 2002). Due to the difference in time scale between variations in \( g_c \) (fast) and \( N \) (slow), \( \lambda \) variations for a given foliar \( N \) content can be assessed without concerns about their joint interactions in the cost function. The marginal N-use efficiency \( \eta \) (see Farquhar et al., 2002) can be defined as

\[ \eta = \frac{\partial A}{\partial N} \]  

(7)

Because the majority of \( N \) is invested in photosynthesis-related proteins, photosynthetic capacity and \( V_{c,max} \) are often tightly correlated with total \( N \) (Evans, 1989). The slope of the \( A-N \) relationship reflects \( N \) investment among various photosynthesis-
related and structural pools in the leaf (Field and Mooney, 1986). It may vary seasonally and with growth conditions such as $c_a$ or light availability (Niinemets and Tenhunen, 1997; Crous and Ellsworth, 2004).

When the range in the observed values of $N$ is wide enough, the $A-N$ and $V_{c,max}-N$ relationships tend to saturate (Evans, 1989). Based on fertilization experiments, this saturation has been attributed to decreasing Rubisco activation state, or $V_{c,max}$/Rubisco ratio (Cheng and Fuchigami, 2000; Warren et al., 2003). Thus, in a given light environment, increasing $N$ availability may not affect the fractional allocation to Rubisco, but more $N$ may be accumulated as photosynthetically inactive ‘storage-Rubisco’. In the following, the ‘photosynthetically active’ $N$ is thus denoted by $N_p$, and the total $N$ expressed on the total needle surface area basis, $N_a$. The most elementary representation of this type of saturation effect is to assume $N_p$ increases proportionally to $N_a$, up to a transition $N_a$, above which $N_p$ remains constant.

Using eqn (3), the formulation for eqn (7) can be expanded in terms of photosynthetic parameters to yield

$$\eta = \frac{\partial A}{\partial N_a} = \frac{\partial A}{\partial a} \frac{\partial a_1}{\partial N_p} + \frac{\partial A}{\partial c_1} \frac{\partial c_1}{\partial N_p} + \frac{\partial A}{\partial T_1} \frac{\partial T_1}{\partial N_p}$$

$$= \frac{\partial N_p}{\partial a} \frac{\partial a_1}{\partial N_p} + \frac{\partial A}{\partial c_1} \frac{\partial c_1}{\partial N_p} + \frac{\partial A}{\partial T_1} \frac{\partial T_1}{\partial N_p}$$

$$= \frac{\partial N_p}{\partial N_a} \left[ \frac{1}{c_1 + a_2} \left( \frac{c_1 - 1}{\partial N_p} + \frac{a_2 + I^*}{c_1 + a_2} \frac{\partial h}{\partial N_p} \right) \right] \approx \frac{1}{c_1 + a_2} \left( \frac{c_1 - 1}{\partial N_p} + \frac{a_2 + I^*}{c_1 + a_2} \frac{\partial h}{\partial N_p} \right)$$

$$= \frac{\partial N_p}{\partial N_a} \left[ \frac{1}{c_1 + a_2} \left( \frac{c_1 - 1}{\partial N_p} + \frac{a_2 + I^*}{c_1 + a_2} \frac{\partial h}{\partial N_p} \right) \right]$$

where $T_1$ accounts for variations in $c_1$ with $g_c$ and $A$, as well as the change in $a_1$ with respect to $N_p$, and $T_2$ accounts for possible variations in $c_1$ originating solely from $N_p$. When a change in $N_p$ causes a smaller relative change in $c_1$ than in $a_1$ (here $V_{c,max}$) the ratio of $T_2$ to $T_1$, expressed as $(\partial c_1/\partial c_a)/(\partial a_1/\partial a_a)$, becomes much smaller than unity. When stomata regulate their aperture to maximize $A$ at given $E$, the $c_1$ at the optimum $g_c$ does not vary with $a_1$ (or $N$) as shown in eqn (5) so that $\partial c_1/\partial N_p = 0$. As a result, eqn (8) reduces to a simpler form that includes $T_1$ only. Hence, when $(\partial c_1/\partial c_a)/(\partial a_1/\partial a_a)$ approaches unity, stomata may not be operating ‘optimally’ in the carbon-gain and water-loss economy, and the joint optimization problem with $N$ included becomes necessary.

In addition to $N$ availability affecting the activation state of Rubisco (through $\partial N_p/\partial N_a$), the activation state may also change with leaf age in response to a decrease in CO$_2$ supply to chloroplasts (Ethier et al., 2006), yet is not affected by $c_a$ (Rogers and Ellsworth, 2002). Elevated $c_a$ may, however, induce changes in $\partial a_1/\partial N_p$ through Rubisco-specific down-regulation. The values of $a_1$, $a_2$, and $I^*$ (eqn 3) as well as $\partial a_1/\partial N_p$ also vary with leaf temperature ($T_1$). We described the dependence of $a_1 = (V_{c,max}$ under saturating light) on $T_1$, based on common formulations such as those in Campbell and Norman (1998), and we modelled $V_{c,max}$ at 25°C as

$$V_{c,max25}(25°C, N_p) \approx s_1 N_p + s_2$$

where $s_1$ and $s_2$ are parameters that describe the sensitivity of $V_{c,max25}$ to $N_p$. The differentiation of eqn (9) with respect to $N_p$ yields $\partial V_{c,max}/\partial N_p \approx s_1(T_1)$, where only the effect of the slope $s_1$ is retained.

**Linking marginal N-use efficiency with marginal water-use efficiency.** Combining the simplified photosynthesis model with the version of eqn (8) accounting for $T_1$ only (i.e. optimal stomatal regulation) results in

$$\eta_{LI} = \frac{c_1}{a_2 + rc_1} \left( \frac{\partial A}{\partial N_p} \right) \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

$$= \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p} \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

$$= \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p} \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

$$= \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p} \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

$$= \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p} \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

$$= \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p} \approx \frac{c_1/c_a}{a_2 + rc_1} \frac{\partial A}{\partial N_p}$$

This expression shows that, when stomata are operating optimally [$\partial f(\delta C)/\partial g_c = 0$], for a given temperature-dependent $V_{c,max}/N_p$ and at a given $D$ and $c_a$, $\eta_{LI}$ and $\lambda_{LI}$ are complementary, as $\eta_{LI}$ increases and $\lambda_{LI}$ decreases with increasing $c_1$ (eqn 5). Increasing $c_a$ will increase both $\lambda_{LI}$ and $\eta_{LI}$. These relationships are explored using the experiments described next.

**Experimental data.** Setting. The Duke FACE experiment is located within a Pinus taeda plantation (established in 1983) in the Blackwood Division of Duke University’s Duke Forest, in Orange County, North Carolina, USA (35°58′N, 79°08′W). Summers are warm and humid and winters are moderate. The mean annual temperature and precipitation are 15.5°C and 1145 mm, respectively. The soil is moderately low-fertility, acidic clay-loam of the Enon series.

This study is based on data collected from the FACE prototype, FACEp (the first elevated CO$_2$ plot and its reference plot) and the replicated FACE experiment (three additional plot pairs) in FACEp, CO$_2$ enrichment started in 1994 (targeted up to 550 μmol mol$^{-1}$), and in 1996 in the three additional elevated FACE plots (targeted at +200 μmol mol$^{-1}$). In 1998, FACEp plots were split in half by an impermeable barrier and one-half of each was fertilized annually. Concurrently, four pairs of 10 m × 10 m ancillary plots were established nearby and one plot of each pair was also fertilized. In 2005, four pairs of 10 m × 10 m ancillary plots were sampled from the upper and lower thirds of the canopy, respectively. The soil is moderately low-fertility, acidic clay-loam of the Enon series.

**Sampling regime.** In each measurement campaign (Table 1), the aim was to sample needles from all four treatments and two canopy layers in as short time as possible. Except for the year 2008, the data from the unfertilized plots from the current dataset are also included in the synthesis paper by Ellsworth et al. (2012). The number of gas-exchange systems used (1–3) and of plot pairs sampled (1–5) varied by campaign. Sun- and shade-acclimated needles were sampled from the upper and lower thirds of the canopy, respectively. Depending on the season, current-year (autumn), 1-year-old needles (spring) or both age classes (summer)
Palmroth et al. — Nitrogen and water-use efficiencies under elevated CO₂

were sampled (Table 1). The central walk-up tower in each of the eight FACE plots, and a triangular tower in each ancillary plot, allowed access to the crowns of 1–5 trees in each treatment. Sampling order was randomized among treatments, plots within treatment, and trees within the plot. When possible, we sampled any individual tree only once in each campaign. When both age classes were measured, however, they were sampled from the same branch.

Gas-exchange measurements. All gas-exchange measurements were made with an open gas-exchange systems (Li-Cor 6400 with 6400-02B red/blue light source, and 20 × 30 mm chamber; Li-Cor Biosciences, Lincoln, NE, USA) on detached shoots (see Maier et al., 2008; Drake et al., 2010). From each sample, we measured a single ‘A–c𝑖’ curve, i.e. the response of A to varying c𝑖, using the following procedure. The midsections of two or three fascicles were inserted in the leaf cuvette, where conditions were maintained at saturating light (1800 μmol m⁻² s⁻¹ PPFD), near ambient temperature, and within a narrow range in D (Table 1). After the gas-exchange rates were stabilized, at growth c_p, E and A were recorded at eight concentrations of c_p, between 60 and 1800 ppm.

After the gas-exchange measurements, needle length (mm) and diameter (mm) were measured to estimate total needle surface area, and the needle area in the chamber was used for rescaling the measured gas-exchange rates. The sampled fascicles were then oven-dried to constant mass at 65 °C (for 48 h), weighed and ground. Leaf mass per unit area (M_A, g m⁻²) was calculated as the ratio of needle dry mass to total surface area. Needle N concentration was determined using a Carlo-Erba analyser (model NA 1500; Fison Instruments, Danvers, MA, USA).

Parameter estimation and other statistical analyses. The Farquhar-model parameters (Farquhar et al., 1980; eqn 3), including V_c,max were estimated from the A–c_i curves following a fitting procedure similar to that described in Ellsworth et al. (2004). Our analysis focused on V_c,max, normalized to a standard temperature, V_c,max25, through the T_L-response function proposed by Campbell and Norman (1998). To minimize the possible bias in the values of the A–c_i curve parameters caused by very low fluxes or a leaky chamber, an A–c_i curve was omitted from the subsequent analysis if (a) the observed g_c changed >30% during the measurements, (b) g_c was <0.03 mol m⁻² s⁻¹, or (c) the intercept of the A–c_i curve was more negative than −2.5 μmol m⁻² s⁻¹.

The simplest approach to account for a saturating response of V_c,max25 to N_A is to assume a piecewise linear relationship, where N_p increases proportionally with N_A, ∂N_p/∂N_A = 1, up to a transition point, after which it remains constant regardless of further increases in N_A. This piecewise representation implies that up to the transition point, the activation state of Rubisco either remains constant or its decrease is compensated by an increase in the fractional allocation to carboxylating enzymes. The transition point was not treated as a free parameter in the fitting, but rather set a priori at the N_A value where the value of the slope in the in the V_c,max25−N_A relationship began to drop.

Estimates of WUE and PNUE were obtained from E and A measured at growth c_p. Marginal resource-use efficiencies were calculated using eqns (5), (8) and (11). For A, the mean D for...
the curve and $c_i$ at growth $c_s$ were used. For $\eta$, the curve-specific Farquhar-model parameters were used, with $r$ set to 0-7 as determined from stable isotope measurements (Ellsworth et al., 2012).

All following analyses rely on the assumption that CO$_2$ exchange is sensitive to fluctuations in stomatal conductance. However, note that the expressions for $V_{c,max}$ and its estimation method applied here are only valid when mesophyll conductance can be assumed to be non-limiting. Mesophyll conductance is partially explained by leaf structure, and studies on conifers (thick, dense leaves; Flexas et al., 2008) suggest that the gas-phase limitations to $A$ are small (<30%) compared with internal limitations. Consequently, our estimated $V_{c,max}$ is better interpreted as a ‘macroscopic’ kinetic constant that also accounts for the internal diffusive limitations of leaves.

We looked for the treatment and age effects on functional relationships ($V_{c,max}$ vs. $N_c$, and $E$ vs. $A$) to identify the smallest number of distinct populations represented by the data. The largest number of possible populations is eight, i.e. two CO$_2$ concentrations, two N treatments and two age classes, and the smallest is one. Based on the extra-sum-of-squares principle (Ramsey and Schaëfer, 1997), a single relationship presents a ‘reduced’ model, and a ‘full’ model includes different parameters for each sub-group. The difference in the mean squared error between full and reduced models was tested ($F$-test). All regressions were estimated using standard general linear models and least-square fitting procedures either in MatLab (MatLab 2009a; MathWorks, Natick, MA, USA) or Systat (Systat Software Inc., Richmond, CA, USA). Because our sampling regime was unbalanced, few statistical tests of the treatment effects on $N_a$ and its dynamics can be performed.

**RESULTS**

Figure 1 shows how the range of foliar N concentrations and light-saturated photosynthetic rates measured (at growth $c_s$ and various leaf temperatures) on leaves of single species and stand in this study relates to observations in a global dataset (Wright et al., 2004). In the first two sampling years $N_a$ of the fertilized current-year needles was 30% higher compared with unfertilized needles in each CO$_2$ treatment (2002–2003; $n = 5$, maximum $P = 0.01$, $t$-test). In 2008, based on a split-plot ANOVA (were $c_s$ is the main effect and N availability the split-plot effect), fertilization increased the mean $N_a$ of 1-year-old needles by 30% ($n = 4$, maximum $P = 0.03$). Finally, elevated $c_a$ did not alter $N_a$ of either age class (minimum $P = 0.51$).

When scaled to a common leaf temperature ($T_L$) of 25°C, the response of $V_{c,max}$ to $N_a$ saturates for the current-year needles (Fig. 2A, B). For the linearly increasing part, where both age classes are presented, the intercept of the regression was lower for the current-year than the 1-year-old needles ($P < 0.01$, ANCOVA). The driving variable was re-scaled to reflect the fraction of $N$ that is photosynthetically active ($N_p$, Fig. 2C), such that when $N_a < 1.4$ g m$^{-2}$, $N_p$ increased linearly with $N_a$ and, for $N_a > 1.4$ g m$^{-2}$, $N_p$ saturates with respect to $N_a$. Moreover, the $V_{c,max} - N_p$ relationship could be described with a single linear regression when $N_p$ of 1-year-old needles was set to 0.9 of that in the current-year needles (Fig. 2D).

We found no fertilization effect on the $V_{c,max} - N_p$ response when evaluated for overlapping ranges in either growth $c_s$ (minimum $P = 0.58$, $F$-test for the difference between mean squared errors of reduced and full regression models). Also, elevated $c_a$ did not affect the $V_{c,max} - N_p$ response in either age class (minimum $P = 0.32$). Taken together, the slope of the $V_{c,max} - N_p$ relationship ($\partial A / \partial N_p$) could be described with a single temperature-dependent function (Fig. 2E). The residuals of the model showed no trends with $N_a$ (Fig. 2F), $T_L$, and $D$ (not shown).

We then assessed possible age and treatment effects on the relationship between $E$ and $A$, and thus, WUE and $\lambda_{EL}$. In Fig. 3, to reduce the dimensions of the analysis, $E$ is plotted as a function of $A_{380} \times D^{1/2}$, where $A_{380}$ is CO$_2$ exchange rate measured at $c_s$ of 380 $\mu$mol mol$^{-1}$ across the treatments, and multiplication by $D^{1/2}$ allows interpreting the slope of the $E$-$A_{380} \times D^{1/2}$ relationship as the inverse of $\sqrt{(\lambda_{EL} c_s)}$ (eqn 6). Transpiration rate was approximately linearly related to
A\textsubscript{380} \times D\textsuperscript{1/2} across the sampled leaves, yet the slope appeared to vary with needle age and N content per unit leaf area. The water loss associated with a given CO\textsubscript{2} uptake was somewhat higher in 1-year-old compared with current-year needles (P < 0.01, F-test). Nitrogen fertilization did not change the E – A\textsubscript{380}D\textsuperscript{1/2} relationship in either needle age class or growth c\textsubscript{a} (minimum P = 0.31, F-test). Nevertheless, when the data for current-year needles were grouped by N\textsubscript{a} (high and low;
Fig. 3B), needles in the high-\(N_a\) group (including sun-acclimated needles across treatments) fixed slightly more \(\text{CO}_2\) at any given \(E\) loss (\(P = 0.01\), \(F\)-test).

PNUE and WUE were inversely correlated (\(P < 0.01\); Fig. 4). To provide a context for this negative correlation, we note that, by definition, PNUE = WUE \((E/N)\). Hence, for a constant \(E/N\), any correlation between PNUE and WUE must be positive. It follows that the inverse correlation between PNUE and WUE must originate from an inverse correlation between variations in \(E\) versus \(N\) implying \(N\) and water trade-off. This trade-off is better revealed when marginal \(N\) and water-use efficiencies are used (Fig. 5A; eqn 11), as these quantities are not affected by the measurement conditions. Note that in Figs 4 and 5, WUE and \(\lambda_{LI}\) were scaled by \(c_a\) to account for the effect of \(c_a\) on \(A\). Complementarity is expected between \(\eta_{LI}\) and the square root of \(\lambda_{LI}\) since both sides of eqn (11) depend on \(c_1\) in opposite ways. A similar dependence of \(\eta\) on \(\lambda_{LI}\) is obtained when the optimality assumption is relaxed and the full version of eqn (8) is used in estimating \(\eta\) (Fig. 5B). The term denoted as \(T_2\) in eqn (8) accounts for possible variations in \(c_1\) originating from \(N_p\) at a given stomatal conductance and was always negative. The ratio \(T_2/T_1\) decreased with increasing \(N_p\) and averaged at \(-0.33\) and \(-0.22\) for needles grown under ambient and elevated \(c_a\), respectively, explaining the downward shift and larger variability in estimates of \(\eta\) (as compared with \(\eta_{LI}\)) at each \(\lambda_{LI}\) (Supplementary Data online).

**DISCUSSION**

In this study, we presented a simplified analytical scaling rule that relates marginal \(N\) and water-use efficiencies (respectively \(\eta_{LI}\) and \(\lambda_{LI}\)), the values of which can be readily derived from measured \(A–c_1\) curves and foliar \(N\). The wide range of \(A\) and \(N\) found among trees grown at Duke FACE allowed the link between \(\eta_{LI}\) and \(\lambda_{LI}\) and the effects of elevated \(\text{CO}_2\) and site \(N\) fertility on the relationship to be characterized.

The mass-based foliar \(N\) concentration \((N_m)\), the leaf-mass-to-area ratio \((M_A)\) and their product, \(N_mm\) vary considerably across biomes, functional types and within a stand (Wright et al., 2004; Fig. 1). The large variability in \(N_m\) and especially \(N_mm\) in our dataset has two sources: the availability of light and \(N\). The physiological implications of variability in these two resources, as reflected in the within-canopy distribution of nitrogen and carbon and among various pools, can be quite different (Ninemets and Tenhunen, 1997). First, we sampled needles from various heights in the canopy to capture the range in \(N_a\) induced by variation in light environment: while \(N_m\) varied little among canopy positions, \(N_a\) decreased as \(M_A\) decreased with decreasing light availability (data not shown). Secondly, the Duke FACE stand is
Our data expands the large dataset of gas-exchange data collected at Duke FACE (Crous et al., 2008; Maier et al., 2008; Ellsworth et al., 2012), and adds to the small amount of data available from the elevated $c_a \times N$ experiments. Regardless of treatment, the rate of change in $V_{c, max}$ with $N_a$ observed in the current study agrees with the earlier data from the unfer-tilized Duke FACE plots (Ellsworth et al., 2012), but extends the range to $N_a > 1.5$ g m$^{-2}$, thus, beyond previously observed values. Also, consistent with previous findings on $P. taeda$ at this site, no acclimation of photosynthesis to elevated $c_a$ was found in current-year needles. For 1-year-old needles, however, Crous et al. (2008) showed a downward shift in the $V_{c, max} - N_a$ response for needles grown under elevated $c_a$, compared with needles grown under ambient $c_a$, but no shift when the trees received additional N. The down-regulation of Rubisco in response to $c_a$ can be accounted for in the present framework as a reduction in $\partial \lambda_{d}/\partial N_a$ and $\eta_{l,l}$. As observed in other studies (DeLucia and Schlesinger, 1991; Cernusak et al., 2008; Han, 2011) PNUE and WUE were inversely related (Fig. 4). The rather large variability in the flux-based efficiency estimates is due to variation in $T_L$ and $D$ thus weakening the expected inverse correlation between them (Wright et al., 2003). However, both PNUE and WUE consistently increased with elevated $c_a$, due to larger $A$ for given $E$ and leaf $N$. Han (2011) studied changes in water and N use with height in the tree (at constant light availability), and found that both $N_m$ and $N_a$ increased, but stomatal conductance decreased with height, and that light-saturated $A$ was inversely correlated with $N_a$. This implies that the limited water available to taller trees (hydraulic limitation) increased the N cost associated with carbon gain and lead to a trade-off between PNUE and WUE. In our dataset, an analysis of the $E-A_{380} \times D^{1/2}$ response (similar to one in Fig. 3) revealed that WUE and $\lambda_{d,l}$ were similar for the upper and lower thirds of the canopy. However, height and light availability co-vary in our study and their effects on stomatal conductance are therefore inseparable.

As predicted by eqn (11), $\eta_{l,l}$ and $\lambda_{d,l}$ were inversely related (Fig. 5A). The values of $\lambda_{d,l}$ and $\eta_{l,l}$ were computed using eqns (5) and (11), therefore assuming that $\partial c_l/\partial N_p = 0$ (i.e. at optimal stomatal conductance). Both $\eta_{l,l}$ and $\lambda_{d,l}$ vary with $c_l$, and, when plotted against each other, the data split into various groups based on growth $c_a$, $N_a$ and needle age. Up to relatively high nitrogen content per unit leaf area ($N_a \leq 1.4$ g m$^{-2}$), $\eta_{l,l}$ and $\lambda_{d,l}^{1/2}$ are complementary, and elevated $c_a$ shifts the relationship upwards. At the higher end of the observed $N_a$, $\eta_{l,l} = 0$ (points not shown in Fig. 5), because of the chosen piecewise linear model that relates changes in $N_p$ to those in $N_a$. This approximation may mask a more realistic scenario where, at a given $c_a$, each additional increase in $N_a$ results in diminishing returns in terms of CO$_2$ uptake. Lastly, due to age-related decline in photosynthetically active $N$, $\eta_{l,l}$ was somewhat, but not consistently, lower in 1-year-old than in current-year needles.
We computed $\eta_i$ first by assuming the needles were operating at their optimal stomatal conductance, i.e. that $c_i$ changes predictably with $D$ but not with $N_a$. To assess the consequences of this assumption, to estimate $\eta_i$ we used the full version of eqn (8), where $T_2$ accounts for possible variations in $c_i$ originating from $N_a$ at a given stomatal conductance (see Fig. 3). $T_2$ becomes important for computing $\eta_i$ if a change in $N_a$ causes a relative change in $c_i$ comparable to the relative change in carboxylation rate ($V_{C_{\text{max}}}$. The results indicate that this may indeed be the case under certain conditions, in particular at high $N_a$. It also implies that the marginal water-use efficiency tends to increase with $N_a$, reflecting a larger $c_{i\text{a}}-c_{i\text{a}}$ gradient and CO$_2$ uptake at a given transpiration rate (Fig. 3B; Supplementary Data). This suggests that stomata may not be operating strictly optimally in terms of carbon-gain and water-loss economy, as predicted by the current objective function. This optimality model does not explicitly include N limitation and may also need to be constrained by leaf structural properties, such as plasticity in leaf mass per area.

Conclusions

On the basis of the optimal stomatal control theory and a linearized CO$_2$-demand function at the photosynthetic site, we obtained an analytical relationship between marginal water and N-use efficiency that implies complementarity between these two quantities. Data collected in a P. taeda canopy supported the model predictions, exhibiting scaling between marginal N- and water-use efficiencies, thus allowing derivation of one quantity from the other. Future work should assess situations where linearized CO$_2$ demand function cannot be assumed. For P. taeda these include lower than saturating light availability and higher atmospheric CO$_2$ than that targeted in the Duke FACE experiment. In search of a better description of the optimization problem plants are facing, the proposed approach can be used to evaluate the generality of these two quantities. Data collected in a mature P. taeda forest can also be used to test these assumptions.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consists of an assessment of the relative importance of Terms 1 and 2 ($T_1$ and $T_2$ in eqn 8) when estimating marginal N-use efficiency.

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LITERATURE CITED


