The Coordination of Leaf Photosynthesis Links C and N Fluxes in C₃ Plant Species

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Abstract

Photosynthetic capacity is one of the most sensitive parameters in vegetation models and its relationship to leaf nitrogen content links the carbon and nitrogen cycles. Process understanding for reliably predicting photosynthetic capacity is still missing. To advance this understanding we have tested across C₃ plant species the coordination hypothesis, which assumes nitrogen allocation to photosynthetic processes such that photosynthesis tends to be co-limited by ribulose-1,5-bisphosphate (RuBP) carboxylation and regeneration. The coordination hypothesis yields an analytical solution to predict photosynthetic capacity and calculate area-based leaf nitrogen content (Nₐ). The resulting model linking leaf photosynthesis, stomata conductance and nitrogen investment provides testable hypotheses about the physiological regulation of these processes. Based on a dataset of 293 observations for 31 species grown under a range of environmental conditions, we confirm the coordination hypothesis: under mean environmental conditions experienced by leaves during the preceding month, RuBP carboxylation equals RuBP regeneration. We identify three key parameters for photosynthetic coordination: specific leaf area and two photosynthetic traits (kₑ, which modulates N investment and is the ratio of RuBP carboxylation/oxygenation capacity (Vₐₙ₀) to leaf photosynthetic N content (Nₐ); and Jmax, which modulates photosynthesis for a given kₑ and is the ratio of RuBP regeneration capacity (Uₘₐₓ) to Vₐₙ₀). With species-specific parameter values of SLA, kₑ and Jmax, our leaf photosynthesis coordination model accounts for 93% of the total variance in Nₐ across species and environmental conditions. A calibration by plant functional type of kₑ and Jmax still leads to accurate model prediction of Nₐ while SLA calibration is essentially required at species level. Observed variations in kₑ and Jmax are partly explained by environmental and phylogenetic constraints, while SLA variation is partly explained by phylogeny. These results open a new avenue for predicting photosynthetic capacity and leaf nitrogen content in vegetation models.

Introduction

The response of leaf net photosynthesis to variations in light, temperature and CO₂ concentration has been successfully represented by the biochemical model of C₃ photosynthesis proposed by Farquhar, von Caemmerer and Berry [1]. This model has pioneered the mechanistic representation of the main biochemical processes of leaf photosynthesis, based on the assumption that photosynthesis is limited by either the carboxylation/oxygenation of ribulose-1,5-bisphosphate (RuBP) by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Vₐₙ₀), or the regeneration of RuBP by the electron transport chain (Hₑ). Maximum rates of these two processes are determined by carboxylation capacity (Vₐₙ₀) and electron transport capacity (Jmax). A strong correlation linearly links the variations of Vₐₙ₀ and Jmax across species (e.g. [2]) and environmental conditions during plant growth (e.g. [3,4]). Since both capacities are measured independently, this result suggests that CO₂ assimilation is regulated in a coordinated manner by these two processes [5].

The variations of net photosynthesis with growth condition, season and species, are related to concurrent changes in leaf nitrogen content (Nₐ) and to the allocation of nitrogen between different protein pools [6]. Vₐₙ₀ and Jmax linearly correlate with Nₐ at both intra-and-inter-specific levels [3,4,7]. Nevertheless, so far the relationship between Vₐₙ₀ and Jmax and their link to Nₐ are empirical correlations, their scatter is substantial, and a predictive process understanding C–N coupling at the leaf scale is still missing. As photosynthetic capacity is among the most influential parameters in current vegetation models [8], such an understanding is essential to predict photosynthesis at leaf, plant, stand and ecosystem scales under changing environmental conditions.

Haxeltine and Prentice [9] suggested a general model for the light-use efficiency of primary production, which links photosynthetic capacity and N. This model is based on the Farquhar’s model of photosynthesis and has been implemented in the global terrestrial vegetation model LPJ [10]. This approach does not account for N limitation and is based on the optimization theory that maximizes assimilation against incoming radiation. Until now, a clear understanding of leaf N variations along vegetative
canopies as well as across species and environments has not been provided by the optimization theory [11,12]. For instance, all reported studies observed N gradients less steep than predicted with the optimization theory, suggesting that it likely overestimates predicted C gain [13–18]. Moreover, there are several limitations in optimization theory calculations (for a detailed discussion, see [19]).

Chen et al. [20] proposed an alternative approach: the coordination hypothesis of leaf photosynthesis. The basic assumption of this approach is that $V_{C_{\text{max}}}$ and $J_{\text{max}}$ are actively regulated by plants in response to environmental conditions such that for most representative conditions $W_c$ equals $W_f$. The optimality criterion in this context is not maximum C gain (as proposed in [21–23]), but the balance of RuBP carboxylation and regeneration, providing a coordinated allocation of resources, i.e. nitrogen, to these two photosynthetic processes (Fig. S1). For vertical gradients within canopies the co-limiting N content was shown to increase with irradiance and to decline with temperature and with atmospheric CO2 concentration [20]. In agreement with experimental studies, the coordination hypothesis showed that N distribution with canopy depth declines less than the light gradient [13–18].

However, so far this co-limitation and its link to $N_c$ has been considered only for vertical gradients within plant canopies, and has not yet been studied and validated across plant species and environmental conditions. This is possibly due to a lack of appropriate data including environmental growth conditions and photosynthetic parameters for a range of C3 plant species. In addition, a full test of this hypothesis requires extending the calculation of the co-limiting N content to account for the coupling between leaf photosynthesis and stomatal conductance [3] as well as ascribing leaf N to structural and metabolic pools [24,25].

In this study, we evaluate for the first time the coordination hypothesis for sunlit leaves and its link to $N_c$ for a large range of plant species grown under different environmental conditions. We use an extended version of the Farquhar model of $C_3$ photosynthesis, a stomatal conductance model and a leaf N model to couple C, N and water fluxes at the leaf scale (see equations and variables in Tables 1–2). We apply this model to a dataset that includes leaf and environmental characteristics during plant growth and gas exchange measurements for a total of 31 $C_3$ species (293 observations, Table S1). For each observation, plant characteristics included the specific leaf area ($SLA$, m$^2$ g$^{-1}$ DM), $N_c$ (gN m$^{-2}$), and $V_{C_{\text{max}}}$ and $J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$) at reference temperature and atmospheric CO2 concentration. The dataset covers six plant functional types (PFTs) grown both under constant and outdoors environments at a range of N and water supplies and atmospheric CO2 concentrations.

In agreement with the half-life time of Rubisco [26], we assumed that photosynthetic coordination varies with the mean over one month of the environmental conditions during plant growth. We tested the coordination hypothesis: i) by comparing simulated $W_f$ and $W_c$ values for the measured $N_c$ and ii) by comparing simulated ($N_c$) and measured ($N_c$) leaf N contents. Second, thanks to a statistical model, we distinguished the plant species and environmental conditions effects on leaf photosynthetic traits. Third, we tested the implications of our leaf photosynthesis coordination model for net C assimilation ($A_n$) and for photosynthetic N use efficiency ($PNUE$) by varying plant photosynthetic traits and environmental growth conditions. Based on these results, we discuss the applicability of the coordination hypothesis to predict photosynthetic capacity and N content of sunlit leaves at the ecosystem and global scales.

## Methods

### A Model Coupling Leaf N with CO2 and H2O Fluxes

Several formulations and parameterizations of the original model by Farquhar et al. [1] have been described. Here, we refer to the formulation and parameterization used by Wohlfahrt et al. [3]. The net rate of C assimilation ($A_n$, µmol m$^{-2}$ s$^{-1}$) was limited either by carboxylase activity of Rubisco ($W_r$, µmolCO2 m$^{-2}$ s$^{-1}$) or by electron flux through the chloroplast photosystems ($W_e$, µmolCO2 m$^{-2}$ s$^{-1}$) (see Eqn 3–4, 7 in Table 1). Their respective capacity, $V_{C_{\text{max}}}$ and $J_{\text{max}}$ scaled with photosynthetic leaf N content ($N_{\text{leaf}}$, µg N m$^{-2}$) (Eqn 6, 9). The relationship between the intracellular CO2 concentration ($C_{\text{c}}$, Pa) and the stomatal conductance ($g_s$, µmol m$^{-2}$ s$^{-1}$) was modeled according to Farquhar et al. [27] (Eqn 14–17). $g_s$ can limit $A_n$, and thereby modify the linearity of the photosynthetic capacities to $N_{\text{leaf}}$ relationship [28]. An analytical method was used to couple $A_n$ and $g_s$, leading to the calculation of $A_n$ through a system of five equations and five unknowns [29,30] (Eqn 17). The daytime temperature dependence of $V_{C_{\text{max}}}$ and $J_{\text{max}}$ was described following Medlyn et al. [31] (Eqn 12). Some studies have shown from a large dataset that the entropy terms of $V_{C_{\text{max}}}$ and $J_{\text{max}}$ acclimate to the mean growth temperature ($T_{\text{g}}$ K) experienced by leaves over the preceding month [32]. The formalism and parameterization proposed by these authors [32] was used in this study to describe the acclimation of $V_{C_{\text{max}}}$ and $J_{\text{max}}$ to $T_{\text{g}}$ (Eq 18–19). Similarly, Ainsworth and Long [33] have shown an acclimation of $A_n$ to atmospheric CO2 concentration during the preceding month ($C_{\text{g}}$ Pa). This was also taken into account (Eqn 20–21), by modifying the relationship of $V_{C_{\text{max}}}$ and $J_{\text{max}}$ at standard temperature ($J_{\text{max},s}$, dimensionless) and the relationship of $V_{C_{\text{max}}}$ at standard temperature to $N_{\text{leaf}}$ ($k_3$, µmolCO2 g$^{-1}$ N s$^{-1}$) according to a linear function of the difference between reference ($C_{\text{g}}$) and growth CO2 concentrations ($C_{\text{g}}$).

A sensitivity analysis of the photosynthesis-stomatal conductance model was performed by analyzing the range of parameter variations in literature (Text S1, Table S2) and the sensitivity of the model outputs in response to a ±15% change in parameter values (Text S1, Fig. S2–S3). An index of sensitivity (IOS) was calculated as the ratio of output to parameter changes and was used to discuss on the model uncertainties linked to model calibration.

### Coordinated N Content of Sunlit Leaves

Within leaves, N is partitioned between metabolic and structural pools [24,25]. The coordinated leaf N content, $N_{\text{leaf}}$ (gN m$^{-2}$) is calculated as the sum of structural leaf N and of photosynthetic leaf N ($N_{\text{phy}}$, gN m$^{-2}$). As leaf structures are highly dependent upon the biomass investment in dry matter (DM) [34], structural leaf N ($f_{\text{h}}$, gN g$^{-1}$ DM) is expressed per unit DM. $f_{\text{h}}$ is assumed constant across species and independent of canopy depth and light intensity. $f_{\text{h}}$ value corresponds to the average value reported in the literature for a range of $C_3$ species (0.012 gN g$^{-1}$ DM, for a review see L ¨otscher et al. [25]. In contrast, metabolic leaf N associated with leaf photosynthesis is expressed per unit area since both light capture and CO2 exchange with atmosphere are intrinsically area-based phenomena [3]. As a key measure of leaf morphology [6], SLA links dry matter-based structural N content ($f_{\text{h}}$) to area-based photosynthetic N content ($N_{\text{phy}}$):

$$N_{\text{ac}} = N_{\text{phy}} + f_{\text{h}}/SLA$$

(1)
Table 1. Equations of the photosynthesis - stomatal conductance models.

| Process | Equation | Unit | Eqn | Ref. |
|---------|----------|------|-----|------|
| Nitrogen sub-model | $N_{\text{leaf}} = N_{\text{leaf}} + f_{\text{leaf}} / \text{SLA}$ | g N m$^{-2}$ | 1 | – |
| | $N_{\text{leaf}} = \frac{4 \times \text{PPFD}}{k_{\text{c}}^2} \left( \frac{C_i + k_2}{(4 C_i + 8 T^3) \Phi_{\text{leaf}}} \right)^2 \left( \frac{1}{f_{\text{leaf}} \Phi_{\text{leaf}}} \right)^{1/2}$ | g N m$^{-2}$ | 2 | – |
| Photosynthetic sub-model | $A_{\text{leaf}} = (1 - \Gamma + C_i) \min \{ W, W \} - R_{\text{day}}$ | µmol m$^{-2}$ s$^{-1}$ | 3 | [1] |
| | $I_{\text{leaf}} = R_{\text{leaf}} / \text{SLA}$ | Pa | 5 | [1] |
| | $V_{\text{c,max}} = k_4 N_{\text{leaf}}$ | µmol m$^{-2}$ s$^{-1}$ | 6 | [2] |
| | $W_i = J_i / 4 C_i + 8 T^3$ | µmol m$^{-2}$ s$^{-1}$ | 7 | [1] |
| | $J = \frac{4 \times \text{PPFD}}{1 + (4 \times \text{PPFD}) / (\Gamma_{\text{max}} \Phi_{\text{leaf}})^3} \left( \frac{1}{f_{\text{leaf}} \Phi_{\text{leaf}}} \right)^{1/2}$ | µmol m$^{-2}$ s$^{-1}$ | 8 | [1] |
| | $J_{\text{max}} = \frac{f_{\text{leaf}}}{\Gamma_{\text{max}} \Phi_{\text{leaf}}}$ | µmol m$^{-2}$ s$^{-1}$ | 9 | [2] |
| | $G_{\text{leaf}} = 0.5 \Omega_i / \Theta_i$ | Pa | 10 | [1] |
| | $R_{\text{day}} = I_{\text{leaf}} \Phi_{\text{leaf}} / \Theta_{\text{leaf}}$ | µmol m$^{-2}$ s$^{-1}$ | 11 | [27] |
| | $I_{\text{leaf}} = 0.5, \text{if } \text{PPFD} > 25 \text{ µmol m}^{-2} \text{s}^{-1}$ | | | |
| | $I_{\text{leaf}} = c \text{PPFD} + d, \text{if } \text{PPFD} \leq 25 \text{ µmol m}^{-2} \text{s}^{-1}$ | | | |
| | $\phi = \exp \left[ \frac{\Delta H_a}{RT} \left( 1 - \frac{T}{T_k} \right) \right] \left( \frac{1}{1 + \exp \left[ \frac{\Delta H_a - \Delta H_d}{RT} \left( 1 - \frac{T}{T_k} \right) \right]} \right)$ | dimensionless | 12 | [31] |
| | $\psi = \exp \left[ \frac{\Delta H_a}{RT} \left( 1 - \frac{T}{T_k} \right) \right]$ | dimensionless | 13 | [27] |
| Stomatal conductance sub-model | $g_s = g_{\text{min}} + g_{\text{ac}} (A_{\text{leaf}} + I_{\text{leaf}} R_{\text{dark}}) 10^3 h_s / C_s$ | mmol m$^{-2}$ s$^{-1}$ | 14 | [27] |
| | $C_s = C_s - A_{\text{leaf}} 10^2 / g_s$ | Pa | 15 | [3] |
| Photosynthesis-stomata coupling | $C_i = C_i - A_{\text{leaf}} 10^2 / g_s$ | Pa | 16 | [29] |
| | $A = a C_i - a d - R_{\text{day}}$ | µmol m$^{-2}$ s$^{-1}$ | 17 | [29] |
| | $e x A_{\text{leaf}} + A_{\text{leaf}}^2 + (e \beta + b \theta + e x R_{\text{day}} - a x) \frac{T}{C_s}$ | | | |
| | $\left( e \gamma + b \frac{T}{C_s} + c \beta R_{\text{day}} + b \theta R_{\text{day}} - a \beta + a d \theta \right) \frac{T}{C_s}$ | | | |
| | $\left( e \gamma R_{\text{day}} + b R_{\text{day}} - a \gamma + a d \theta \right) \frac{T}{C_s}$ | | | |
| | $\phi = g_{\text{min}} / g_{\text{ac}} - g_{\text{ac}} h_s 10^2 + 1.6 10^2$ | | | |
| | $\beta = C_s (g_{\text{ac}} g_{\text{ac}} h_s - 2 g_{\text{min}} - 1.6 g_{\text{ac}} - R_{\text{day}} g_{\text{ac}} h_s 10^2)$ | | | |
| | $\gamma = C_s (g_{\text{ac}} g_{\text{min}} 10^{-2} + R_{\text{day}} g_{\text{ac}} g_{\text{ac}} h_s)$ | | | |
| | $\theta = g_{\text{ac}} g_{\text{ac}} h_s - g_{\text{min}}$ | | | |
| Photosynthetic acclimation | $\Delta S_{\text{leaf}} = \Delta S_{\text{leaf}} + p_4 \left( T - T_o \right)$ | Photosynthetic | 18a | [32] |
| | $J_{\text{leaf}} = J_{\text{leaf}} + p_1 \left( T - T_o \right)$ | J K$^{-1}$ mol$^{-1}$ J K$^{-1}$ mol$^{-1}$ dimensionless | 19 | |
| | $J_{\text{leaf}} = J_{\text{leaf}} + p_2 (C_s - C_k) k_T^2 = k_5 + p_3 (C_s - C_k)$ | dimensionless | 20 | 21 | [33] |

Under given environmental conditions, $N_{\text{leaf}}$ is defined as the $N_{\text{leaf}}$ value at which $A_{\text{leaf}}$ was co-limited by $W_i$ and $W_j$ (Fig. S1). Both $V_{\text{c,max}}$ and $J_{\text{max}}$ are linear functions of $N_{\text{leaf}}$ and, for given environmental conditions, there is a single $N_{\text{leaf}}$ value for which $W_i$ equals $W_j$. At this co-limiting point, $N_{\text{leaf}}$ equals (see Text S2 Eqn 2a-2d for details):
Table 2. Parameters and variables of the photosynthesis - stomatal conductance models.

| Symbol | Value | Unit | Description |
|--------|-------|------|-------------|
| **Parameters** | | | |
| C | −0.02 | m² s μmol⁻¹ | Slope of the linear relationship between \( f_{\text{ac}} \) and PPFD in the range 0–25 μmol m⁻² s⁻¹ |
| \( C_a^{r} \) | 35 | Pa | Reference atmospheric CO₂ partial pressure |
| d | 1 | μmol CO₂ m⁻² leaf s⁻¹ | y-intercept of the linear relationship between \( f_{\text{ac}} \) and PPFD in the range from 0–25 μmol m⁻² s⁻¹ |
| \( g_{\text{h}} \) | 300 | mmol m⁻² s⁻¹ | Leaf boundary layer conductance to water vapour |
| \( g_{\text{fac}} \) | 13.7 | dimensionless | Stomatal sensitivity coefficient |
| \( g_{\text{min}} \) | 76.2 | mmol m⁻² s⁻¹ | Minimum stomatal conductance to water vapour |
| \( h_{\text{ac}} \) | 0.5 | dimensionless | Coefficient representing the extent to which \( R_{\text{dark}} \) is inhibited in the light |
| \( J_{\text{fac}}^{r} \) | dimensionless | Ratio between \( J_{\text{max}} \) and \( V_{\text{Cmax}} \) of plant grown at the reference temperature and at the reference CO₂ partial pressure |
| \( k_{\text{s}} \) | 19.42 | Pa | Michaelis-Menten constant for carboxylase activity of Rubisco |
| \( K_{\text{a}} \) | 14 300 | Pa | Michaelis-Menten constant for oxgenase activity of Rubisco |
| \( O_{\text{a}} \) | 21 000 | Pa | Internal leaf oxygen concentration |
| \( p_{\text{1}} \) | −0.012 | dimensionless | Coefficient representing the extent to which \( J_{\text{fac}} \) is modified by the CO₂ partial pressure during plant growth |
| \( p_{\text{2}} \) | 0.036 | dimensionless | Coefficient representing the extent to which \( J_{\text{fac}} \) is modified by the temperature during plant growth |
| \( p_{\text{3}} \) | 0.3192 | μmol CO₂ g⁻¹ N s⁻¹ | Coefficient representing the effect of CO₂ partial pressure during plant growth on \( k_{\text{s}} \) |
| \( p_{\text{4}} \) | 0.94 | dimensionless | Coefficient representing the effect of growth temperature on entropy term for \( J_{\text{max}} \) and \( V_{\text{Cmax}} \) |
| \( R \) | 8.314 | J K⁻¹ mol⁻¹ | Perfect gas constant |
| \( R_{\text{fac}} \) | 0.11 | dimensionless | Ratio between \( R_{\text{dark}} \) and \( V_{\text{Cmax}} \) at reference temperature |
| \( \text{SLA} \) | m² leaf g⁻¹ DM | Specific leaf area |
| \( \gamma \) | 0.05 | mol CO₂ mol⁻¹ photon | Apparent quantum yield of net photosynthesis at saturating CO₂ |
| \( \Delta H_{\text{H}_{\text{max}}} \) | 83 608 | J mol⁻¹ | Activation energy of \( J_{\text{max}} \) |
| \( \Delta H_{\text{H}_{\text{ac}}} \) | 65 800 | J mol⁻¹ | Activation energy of \( K_{\text{a}} \) |
| \( \Delta H_{\text{K}_{\text{a}}} \) | 36 000 | J mol⁻¹ | Activation energy of \( K_{\text{a}} \) |
| \( \Delta H_{\text{H}_{\text{dark}}} \) | 50 861 | J mol⁻¹ | Activation energy of \( R_{\text{dark}} \) |
| \( \Delta H_{\text{V}_{\text{Cmax}}} \) | 86 529 | J mol⁻¹ | Activation energy of \( V_{\text{Cmax}} \) |
| \( \Delta H_{\gamma} \) | −28 990 | J mol⁻¹ | Activation energy of \( \gamma \) |
| \( \Delta H_{\text{d}} \) | 200 000 | J mol⁻¹ | Deactivation energy |
| \( JS_{\text{max}} \) | 660.42 | J K⁻¹ mol⁻¹ | Entropy term of \( J_{\text{max}} \) for plant grown at reference temperature |
| \( JS_{\text{Cmax}} \) | 654.24 | J K⁻¹ mol⁻¹ | Entropy term of \( V_{\text{Cmax}} \) for plant grown at reference temperature |
| \( \tau \) | 2 838 | dimensionless | Rubisco specificity factor at reference temperature |
| **Input Variables** | | | |
| \( C_{\text{a}} \) | Pa | CO₂ partial pressure in the ambient air |
| \( C_{\text{g}} \) | Pa | Atmospheric CO₂ partial pressure during preceding month of plant growth |
| \( h_{\text{s}} \) | dimensionless | Leaf surface relative humidity |
| PPFD | μmol m⁻² s⁻¹ | Photosynthetic photon flux density |
| \( T_{\text{a}} \) | K | Air temperature. In our analysis \( T_{\text{a}} = T_{\text{g}} \) |
| \( T_{\text{g}} \) | K | Mean air temperature during preceding month of plant growth |
| \( T_{r} \) | 293.16 | K | Reference temperature for metabolic activity |
| **Output variables** | | | |
| \( A_{n} \) | μmol m⁻² s⁻¹ | Net photosynthesis |
| \( C_{\text{i}} \) | Pa | Internal CO₂ partial pressure |
| \( C_{\text{s}} \) | Pa | Leaf surface CO₂ partial pressure |
| \( g_{\text{s}} \) | mmol m⁻² s⁻¹ | Stomatal conductance to water vapor |
| \( K_{2} \) | Pa | Intermediate variable synthesizing the Rubisco affinity for CO₂ |
| \( J \) | μmol m⁻² s⁻¹ | Light dependence of the rate of electron transport |
### Table 2. Cont.

| Symbol | Value | Unit | Description |
|--------|-------|------|-------------|
| $J_{\text{max}}$ | dimensionless | $J_{\text{max}}$ acclimated to CO₂ | CO₂ | 1
| $J_{\text{max}}$ | dimensionless | $J_{\text{max}}$ acclimated to temperature | CO₂ | 1
| $J_{\text{max}}$ | dimensionless | $J_{\text{max}}$ acclimated to CO₂ and to temperature | CO₂ | 1
| $J_{\text{max}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Potential rate of RuBP regeneration | CO₂ | 1
| $J_{\text{max}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Potential rate of RuBP regeneration at reference temperature | CO₂ | 1
| $k_{1}$ | $\mu$mol CO₂ g$^{-1}$ N s$^{-1}$ | Slope of linear relationship relating $N_{\text{pa}}$ to $V_{\text{C}_{\text{max}}}$ | CO₂ | 1
| $k_{1}^{\text{ac}}$ | $\mu$mol CO₂ g$^{-1}$ N s$^{-1}$ | Slope of linear relationship relating $N_{\text{pa}}$ to $V_{\text{C}_{\text{max}}}$ | CO₂ | 1
| $N_{k}$ | g N m$^{-2}$ leaf | Leaf N content per leaf area | CO₂ | 1
| $N_{\text{ac}}$ | g N m$^{-2}$ leaf | Leaf N content per leaf area when $W_{i}$ equals $W_{j}$ | CO₂ | 1
| $n_{\text{pa}}$ | g N m$^{-2}$ leaf | Leaf photosynthetic N content per leaf area | CO₂ | 1
| $n_{\text{pa}}$ | g N m$^{-2}$ leaf | Leaf photosynthetic N content per leaf area when $W_{i}$ equals $W_{j}$ | CO₂ | 1
| $R_{\text{dark}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Leaf dark respiration rate | CO₂ | 1
| $R_{\text{dark}}^{\text{ac}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Leaf dark respiration rate at reference temperature | CO₂ | 1
| $R_{\text{day}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Leaf respiration rate from processes other than photorespiration | CO₂ | 1
| $V_{\text{C}_{\text{max}}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Maximum carboxylation rate of Rubisco | CO₂ | 1
| $V_{\text{C}_{\text{max}}}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Maximum carboxylation rate of Rubisco at reference temperature in the absence of any deactivation as a result of high temperature | CO₂ | 1
| $W_{c}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | Rubisco-limited photosynthetic rate | CO₂ | 1
| $W_{i}$ | $\mu$mol m$^{-2}$ s$^{-1}$ | RuBP regeneration limited photosynthetic rate through electron transport | CO₂ | 1
| $\Phi$ | dimensionless | Temperature dependence of $J_{\text{max}}$ or $V_{\text{C}_{\text{max}}}$ | CO₂ | 1
| $\Phi_{\text{C}_{\text{max}}}$ | dimensionless | Temperature dependence of $V_{\text{C}_{\text{max}}}$ | CO₂ | 1
| $\Phi_{J_{\text{max}}}$ | dimensionless | Temperature dependence of $J_{\text{max}}$ | CO₂ | 1
| $\theta$ | dimensionless | Temperature dependence of $k_{1}$ | CO₂ | 1
| $\theta_{k_{1}}$ | dimensionless | Temperature dependence of $k_{1}$ | CO₂ | 1
| $\theta_{k_{1}}$ | dimensionless | Temperature dependence of $k_{1}$ | CO₂ | 1
| $\theta_{\text{ac}}$ | dimensionless | Temperature dependence of $\tau$ | CO₂ | 1
| $\theta_{\text{Rmax}}$ | dimensionless | Temperature dependence of $R_{\text{dark}}$ | CO₂ | 1
| $\Gamma^{*}$ | dimensionless | CO₂ compensation point in the absence of mitochondrial respiration | CO₂ | 1
| $\Delta S^{a}$ | J K$^{-1}$ mol$^{-1}$ | Entropy term acclimated to temperature during plant growth | CO₂ | 1
| $\Delta S_{\text{Rmax}}^{a}$ | J K$^{-1}$ mol$^{-1}$ | Entropy term of $J_{\text{max}}$ acclimated to temperature during plant growth | CO₂ | 1
| $\Delta S_{\text{C}_{\text{max}}}$ | J K$^{-1}$ mol$^{-1}$ | Entropy term of $V_{\text{C}_{\text{max}}}$ acclimated to temperature during plant growth | CO₂ | 1

Parameter values are derived from Wohlfahrt et al. [3–4].

doi:10.1371/journal.pone.0038345.t002

\[
N_{\text{pa}} = \frac{4.12 \times \text{PPFD}}{k_{3}^{	ext{ac}}} \left( \frac{C_{i} + k_{2}}{(4C_{i} + 8\Gamma^{*}) \Phi_{\text{C}_{\text{max}}}} \right)^{2} - \left( \frac{1}{J_{\text{fac}} \Phi_{J_{\text{max}}}} \right)^{1/2}
\]

where $\alpha$ (mol CO₂ mol$^{-1}$ photon) is the apparent quantum yield of $A_{c}$ at saturating CO₂, $\text{PPFD}$ (μmol m$^{-2}$ s$^{-1}$) is the photosynthetic photon flux density, $k_{3}^{	ext{ac}}$ (μmol CO₂ g$^{-1}$ N s$^{-1}$) is $k_{3}$ acclimated to $C_{s}$ (Eqn 21), $k_{2}$ (Pa) is an intermediate variable synthesizing the Rubisco affinity for CO₂ (Eqn 5), $\Gamma^{*}$ (Pa) is the CO₂ compensation point in the absence of mitochondrial respiration, $J_{\text{fac}}$ is $J_{\text{ac}}$ acclimated to $C_{s}$ and $T_{\theta}$ (CO₂ air concentration and temperature during preceding month of plant growth, Eqn 19–20), and $\Phi_{\text{C}_{\text{max}}}$ and $\Phi_{J_{\text{max}}}$ (dimensionless) are the response functions of $V_{\text{C}_{\text{max}}}$ and $J_{\text{max}}$ to temperature (Eqn 12). Overall, $N_{\text{pa}}$ integrates the sensitivity of photosynthetic machinery to $T_{\theta}$, PPFD, C$$_{s}$ and $h$.

### Dataset

A dataset was assembled from measurements and literature to associate leaf photosynthetic traits of mature sunlit leaves with environmental growth conditions (Dataset S1). $V_{\text{C}_{\text{max}}}$ and $J_{\text{max}}$ at reference temperature ($T_{\theta} = 20$°C), $N_{\text{pa}}$, $\text{SLA}$, as well as $T_{\theta}$, PPFD, $h$, and $C_{s}$ during the month preceding leaf measurements were included. $V_{\text{C}_{\text{max}}}$ and $J_{\text{max}}$ values were standardized using a consistent formulation and parameterization of $\Gamma^{*}$ and the Michaelis-Menten constants for carboxylation ($K_{c}$, Pa) and oxygenase ($K_{o}$, Pa) Rubisco activity [32,33].

The dataset has 293 entries from 31 C$_{3}$ plant species covering six plant functional types (PFTs): temperate broadleaved and coniferous evergreen trees (PFT1), temperate broadleaved deciduous trees (PFT2), deciduous shrubs and herbs (PFT3), perennial C$_{3}$ grasses and forbs (PFT4), C$_{5}$ crops (wheat, wheat) and N-fixing...
trees (PFT6). The final dataset covers a wide range of plant growth conditions: \( T_E \) (ranging from 7.1 to 21.0°C); \( PPFD \) (300 to 1170 μmol m\(^{-2}\) s\(^{-1}\)); \( h_c \) (0.51 to 0.89) and \( C_g \) (36 and 60 Pa). However, data corresponding to severe drought and/or to very low \( N \) availability during growth were excluded from the dataset. Four categories of inorganic \( N \) availability (low, medium, high and very high), two categories of soil moisture and of atmospheric CO\(_2\) concentration (ambient and elevated) and six categories of experimental set-up (climate chamber, sunlit climate chamber, botanical garden, natural vegetation, free air CO\(_2\) enrichment (FACE) and open top chambers) were defined. The dataset has been made available via the TRY initiative on plant traits [36].

Data Analysis

**Coordinated \( W_c \) and \( W_j \).** The basic assumption of the coordination hypothesis is that under the environmental conditions to which a leaf is adapted, RuBP carboxylation equals RuBP regeneration \( (W_c = W_j) \). Here we tested this for the average daily plant growth conditions (excluding night values) during the last month preceding photosynthesis measurements. We used four environmental variables \( (C_g, PPFD, T_E \) and \( h_c) \) corresponding to the average plant growth conditions as model input, and \( V_{\text{max}} \) and \( J_{\text{max}} \) derived from separate photosynthesis measurements on the same plants. A single set of values was used for all other 33 model parameters and was originanted from Wohlfarth’s calibration (Table 2) [3,4]. \( W_c \) and \( W_j \) both predicted for the average plant growth conditions for each observation \( (n = 293) \), were compared by least square linear regression. Regression residuals were analyzed using a general linear model (GLM) with \( T_E \) and \( C_g \) and with PFTs and \( N \) categories. PFTs and \( N \) levels were compared by the post ANOVA Tukey’s HSD method.

**Prediction of the coordinated leaf \( N \) content.** \( N_{\text{ac}} \) was calculated for each observation \( (n = 293) \), using four environmental variables \( (C_g, PPFD, T_E \) and \( h_c) \) corresponding to the growth conditions of the past month and three leaf traits \( (k_3, J_{\text{line}} \) and \( \text{SLA}) \). \( k_3 \) is calculated as the ratio between \( V_{\text{max}} \) and \( N_{\text{ac}} \), while \( J_{\text{line}} \) is calculated as the ratio between \( J_{\text{max}} \) and \( V_{\text{max}} \). The prediction of \( N_{\text{ac}} \) was evaluated by the relative root mean squared error (RRMSE), which is the relative average of the squared differences between predicted and observed values [37]. RRMSE values lower than 0.2 indicate acceptable errors. Systematic (RRMSE\(_{\text{sys}}\)) and unsystematic (RRMSE\(_{\text{uys}}\)) errors [37] specified the error source of RRMSE (Eq. 1).

\[
\text{RRMSE}_{\text{sys}} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (E_i - M_i)^2} / M \quad \text{with} \quad E_i = b \cdot M_i + a
\]

\[
\text{RRMSE}_{\text{uys}} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (E_i - \bar{E})^2} / M \quad \text{with} \quad E_i = b \cdot M_i + a
\]

where \( E_i \) and \( M_i \) are the predicted and measured values of the observation \( i \), \( M_i \) is the average of \( M_i \) and \( \bar{E} \) is an estimate of \( E_i \) derived from the linear regression between \( E_i \) and \( M_i \).

**Dependence of leaf photosynthetic parameters on plant functional type (PFT).** ANOVA followed by LSD method for mean comparison tests, were used to analyze the role of PFT for the estimation of leaf photosynthetic traits used in the test of the coordination hypothesis \( (V_{\text{max}}, J_{\text{max}}, k_3, J_{\text{line}} \) and \( \text{SLA}) \). In order to test if the calibration of leaf photosynthetic traits can be simplified to obtain a unique value or a value by PFT, we estimated independent values of \( k_3, J_{\text{line}} \) and \( \text{SLA} \) traits minimizing the squared differences between \( N_{\text{ac}} \) and \( N_{\text{ac}} \) (Newton’s optimization method). Mean and optimized values per PFT were then compared by linear regressions. The calibration of leaf traits by species was not tested since the number of observations per species was too variable in our dataset.

**Dependence of leaf photosynthetic parameters on environmental growth conditions.** Multiple regression models were used to analyze the effects of environmental growth conditions \( (T_E, PPFD, h_c \) and \( C_g \)) and \( \text{SLA} \) on leaf traits \( (V_{\text{max}}, J_{\text{max}}, k_3, J_{\text{line}} \) and \( \text{SLA}) \). For regression models of \( k_3 \) and \( J_{\text{line}} \), the values of dependent variables were log-transformed and all residuals followed a normal distribution.

We tested if the prediction of leaf photosynthetic traits by environmental growth conditions was robust and validated likewise the coordination hypothesis. We conducted bootstrap analyses to predict \( W_c \) and \( W_j \) as a function of \( V_{\text{max}} \) and \( J_{\text{max}} \) estimated by an independent regression model and environmental growth conditions. In the same way, bootstrap analyses were conducted to predict \( N_{\text{ac}} \) as a function of estimated \( k_3 \) and \( J_{\text{line}} \).

We also attempted to falsify the testable hypothesis \( (W_{i} = W_{j} \) and \( N_{\text{ac}} = N_{\text{ac}} \)) provided by the photosynthetic coordination hypothesis. To this end, we randomized environmental growth conditions among observations (permutation test) and tested the alternative hypothesis significant differences between \( W_{i} \) and \( W_{j} \) and between \( N_{\text{ac}} \) and \( N_{\text{ac}} \).
Predicted Coordinated Leaf N Content ($N_{ac}$) Matches Observed Leaf N Content ($N_a$)

Overall, predicted and observed $N_a$ values were closely correlated with a slope not significantly different from one and an intercept not significantly different from zero (Fig. 1B, $n = 293$, $P < 0.001$, RMSE = 0.12). The breakdown of RMSE into unsystematic and systematic error terms showed that the prediction error was mostly unsystematic and therefore associated to data and not to a systematic model error [RMSEs = 0.012; RMSE0 = 0.108]. An ANOVA on the residuals of the prediction showed weak but significant effects of PFTs, $T_g$ and $k_3$ ($df = 5, 1, 1$, respectively; $P < 0.01$; data not shown).

As $f_{ns}$ was assumed constant across species [25], we calculated $N_{ac}$ and $N_{pac}$ by subtracting the ratio $f_{ns}/SLA$ to $N_a$ and $N_{ac}$, respectively. Similarly, predicted and observed $N_p$ values were closely correlated (Fig. 1C, $n = 293$, $P < 0.001$, RMSE = 0.21).
As carbon content in leaves was assumed to be approximately constant, we calculated a C/N ratio by dividing $N_a$ and $N_{ac}$ by the ratio between a common carbon content ($f_c = 0.45 \text{ g C g}^{-1} \text{ DM}$; [36,38]) and SLA. Predicted C/N matched significantly the calculated C/N, observed across environmental conditions and across species and PFTs (Fig. 1D).

### Dependency of Leaf Parameters on Plant Functional Type

In the dataset (Table S1), the parameters used to calculate leaf photosynthesis and stomatal conductance were $SLA$, $J_{fac}$, $k_3$, calculated from $V_{Cmax}$, $J_{max}$ and leaf N measurements (Eqn 12, 15). At $T^*$, $V_{Cmax}$ and $J_{max}$ varied between $4–141 \text{ mol m}^{-2} \text{ s}^{-1}$ and $8–213 \text{ mol m}^{-2} \text{ s}^{-1}$, respectively. $k_3$ varied from 4.6 to 350 $\text{ mol g}^{-1} \text{ N s}^{-1}$ while $J_{fac}$ values were very constrained from 1.69 to 3.71, as already observed [2]. Finally, $SLA$ varied from 1.5 to 43.2 $\text{ m}^2 \text{ kg}^{-1} \text{ DM}$. All photosynthetic traits showed significant dependency to PFT ($P<0.001$) but with different determination coefficient ($r^2 = 0.66$, 0.64, 0.24, 0.47 and 0.40 for $V_{Cmax}$, $J_{max}$, $k_3$, $J_{fac}$ and $SLA$, respectively). Post-ANOVA LSD tests showed that the discrimination among the PFTs was even more effective for $J_{fac}$, $J_{max}$ and $SLA$ separating significantly four groups among the six PFTs (Table S7) and was much weaker for $k_3$ and $V_{Cmax}$ (two groups were significantly distinguished).

$k_3$, $J_{fac}$ and $SLA$ can be optimized to a value which minimizes the squared differences between $N_a$ and $N_{ac}$ (two groups were significantly distinguished). $k_3$, $J_{fac}$ and $SLA$ can be optimized to a value which minimizes the squared differences between $N_a$ and $N_{ac}$ (two groups were significantly distinguished). When $k_3$ was optimized by PFT, $N_a$ was accurately predicted (slope = 0.96, $r^2 = 0.73$, RRMSE = 0.23). When a single value was used for the whole dataset, $N_a$ prediction was not satisfactory. The optimization by PFT of $J_{fac}$ led to a strong prediction of $N_a$ (slope not different from one, $r^2 = 0.79$, RRMSE = 0.23). When a single value was used for the entire dataset ($J_{fac}$ = 2.11), the prediction of $N_a$ was less accurate but the slope of the relationship between $W_j$ and $W_i$ remained close to one. Finally, the optimisation of $SLA$ by PFT or to a single value for the entire dataset strongly reduced the accuracy of $N_a$ prediction. Optimization of the $k_3$ and $J_{fac}$ parameters showed that $N_a$ can be acceptably predicted when their values are defined by PFT. For all traits, average values by PFT and optimized values by PFT displayed significant linear relationships (Table 3B).

### Table 3. Estimates of the optimized value (for the entire dataset and by PFT) of leaf photosynthetic traits ($J_{fac}$, $k_3$ and SLA).

| A) | Optimized value | $W_i/W_j$ | $N_a/N_{ac}$ |
| --- | --- | --- | --- |
| Parameter | $k_3$ | Slope | $r^2$ | Slope | $r^2$ | RRMSE |
| All | 48.3 | 1.15±0.02 | 0.78 | 0.94±0.02 | 0.64 | 0.28 |
| PFT | 45.2; 37.1; 54.0; 79.4; 46.2; 24.2 | 1.08±0.02 | 0.88 | 0.96±0.02 | 0.73 | 0.23 |
| $J_{fac}$ | All | 2.11 | 1.06±0.02 | 0.89 | 0.97±0.02 | 0.68 | 0.31 |
| PFT | 2.11; 2.11; 2.59; 1.70; 2.33; 3.10 | 1.04±0.02 | 0.92 | 1.02±0.02 | 0.79 | 0.23 |
| SLA | All | 17.7 | 1.02±0.02 | 0.92 | 0.88±0.02 | 0.43 | 0.44 |
| PFT | 8.1; 13.7; 18.2; 20.0; 18.3; 13.4 | 1.02±0.02 | 0.92 | 0.96±0.02 | 0.48 | 0.37 |
| $k_3$ and $J_{fac}$ | All | $k_3 = 48.3; J_{fac} = 2.11$ | 1.18±0.02 | 0.79 | 0.89±0.02 | 0.68 | 0.33 |
| PFT | $k_3 = 45.2; 37.1; 54.0; 79.4; 46.2; 24.2 \ W_i \ J_{max} = 2.11; 2.11; 2.59; 1.70; 2.33; 3.10$ | 1.06±0.02 | 0.88 | 0.96±0.02 | 0.74 | 0.26 |
| B) | $k_3$ | $J_{fac}$ | SLA |
| PFT | Mean | Optimized | Mean | Optimized | Mean | Optimized |
| PFT1 | 65.0 | 45.2 | 2.23 | 2.11 | 11.1 | 8.1 |
| PFT2 | 46.6 | 37.1 | 2.32 | 2.11 | 13.1 | 13.7 |
| PFT3 | 90.1 | 54.0 | 2.53 | 2.59 | 21.4 | 18.2 |
| PFT4 | 86.1 | 79.4 | 2.04 | 1.7 | 22.0 | 20.0 |
| PFT5 | 44.9 | 46.2 | 2.69 | 2.33 | 18.3 | 18.3 |
| PFT6 | 38.1 | 24.2 | 2.50 | 3.1 | 20.3 | 13.4 |
| Correlation | $r^2 = 0.68$ | $P<0.001$ | $r^2 = 0.49$ | $P<0.001$ | $r^2 = 0.68$ | $P<0.001$ |

The squared difference between measured $N_a$ and predicted $N_{ac}$ values were minimized by Newton’s method. A) The optimization was done with one trait at a time without changing the values of the two other traits. The optimized values are ordered by PFT (i.e. the first value corresponds to PFT1). B) The optimized values by PFT were compared to mean per PFT in the dataset by using a linear regression model. Abbreviations: PFT1, temperate broadleaved and coniferous evergreen trees; PFT2, temperate broadleaved deciduous trees; PFT3, deciduous shrubs and herbs; PFT4, perennial C3 grasses and forbs; PFT5, C3 crops (wheat); PFT6, N-fixing trees. doi:10.1371/journal.pone.0038345.t003
### Table 4. Effects of environmental conditions on the leaf photosynthetic traits: $V_{C_{\max}}$, $J_{\text{max}}$, $J_{\text{acr}}$, $k_3$ and SLA.

| A) | Factors | d.f. | Variance | $P$-value | Variance | $P$-value | Variance | $P$-value | Variance | $P$-value | SLA |
|----|---------|------|----------|----------|----------|----------|----------|----------|----------|----------|-----|
|    | $I_{\text{max}}$ | | | | | | | | | | |
| $CO_2$ level | 1 | . | ns | 4.6 | <0.01 | 27.0 | <0.001 | . | ns | . | ns |
| N level | 3 | 35.5 | <0.001 | 24.5 | <0.001 | 9.8 | <0.05 | 65.1 | <0.001 | 7.3 | <0.05 |
| $H_2O$ level | 1 | 12.2 | <0.001 | 15.3 | <0.001 | 8.1 | <0.01 | . | ns | 3.1 | <0.01 |
| PPFD | 1 | 6.6 | <0.01 | 8.9 | <0.001 | 5.7 | <0.05 | 2.1 | <0.05 | 0.1 | <0.01 |
| $T_g$ | 1 | 9.5 | <0.01 | 33.1 | <0.001 | . | ns | 25.3 | <0.001 | 77.9 | <0.001 |
| $h_t$ | 1 | 12.7 | <0.001 | 5.4 | <0.01 | 19.2 | <0.001 | 4.5 | <0.01 | 1.8 | <0.05 |
| PPFD$^*T_g$ | 1 | 5.4 | <0.05 | . | ns | 6.0 | <0.05 | . | ns | . | ns |
| PPFD$^*h_t$ | 1 | 18.1 | <0.001 | 8.2 | <0.001 | 24.2 | <0.001 | 3.0 | <0.05 | 9.7 | <0.05 |
| Overall | 293 | $r^2 = 0.64$ | <0.001 | $r^2 = 0.66$ | <0.001 | $r^2 = 0.51$ | <0.001 | $r^2 = 0.44$ | <0.001 | $r^2 = 0.15$ | <0.01 |

| B) | Factors | Estimate | Error | Estimate | Error | Estimate | Error | Estimate | Error | Estimate | Error |
|----|---------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|
| Constant | $-2.1$ | 12 | $-19.0$ | 42 | 62.7 | E–03 | 257.0 | E–03 | 2.53 | 1.06 | 54.0 | 15.3 |
| $CO_2$ | ns | ns | $-8.2$ | 3.1 | 84.4 | E–03 | 17.4 | E–03 | ns | ns | ns | ns |
| N | 10.4 | 3.3 | 4.1 | 1.2 | $-4.4$ | E–03 | 6.8 | E–03 | $-0.29$ | 0.03 | 0.95 | 0.42 |
| $H_2O$ | $-43.2$ | 13.0 | $-17.8$ | 4.0 | $-65.2$ | E–03 | 26.7 | E–03 | ns | ns | 4.1 | 1.4 |
| PPFD | 0.58 | 0.16 | 0.24 | 0.05 | 0.93 | E–03 | 0.33 | E–03 | 2.77 | E–03 | 1.37 | E–03 | $-0.05$ | 0.02 |
| $T_g$ | $-21.3$ | 7.0 | $-3.0$ | 0.4 | ns | ns | $-59.9$ | E–03 | 6.76 | E–03 | $-0.83$ | 0.13 |
| $h_t$ | 784 | 181 | 210 | 57 | 1.64 | 0.38 | 4.33 | 1.41 | $-41.6$ | 20.9 |
| PPFD$^*T_g$ | 0.018 | 0.008 | ns | ns | 3.62 | E–05 | 1.75 | E–05 | ns | ns | ns | ns |
| PPFD$^*h_t$ | $-1.21$ | 0.24 | $-0.31$ | 0.07 | $-2.4$ | E–03 | 0.5 | E–03 | $-4.01$ | E–03 | 1.81 | E–03 | 0.06 | 0.03 |

The factors are environmental growth conditions: radiation (PPFD), temperature ($T_g$), relative humidity ($h_t$), air CO2 concentration ($CO_2$ level), soil N availability (N level) and soil moisture ($H_2O$ level). A) Degree of freedom (d.f.), variance explained (%), statistical significance and sign (positive or negative) of interactions with continuous variables. B) Coefficients estimate of ANOVA model. All variable values were analyzed at a reference temperature of 20°C. Residuals of analysis followed a normal distribution without transformation for $V_{C_{\max}}$ and $J_{\text{max}}$ and with log-transformation for $J_{\text{acr}}$ and $k_3$. We only included in the ANOVA model the interactions that were significant.

doi:10.1371/journal.pone.0038345.t004

### Dependency of Leaf Parameters to Environmental Growth Conditions

All leaf photosynthetic parameters could be predicted from environmental growth conditions (Table 4). However, SLA was poorly correlated with environmental conditions ($r^2 = 0.15$). $I_{\text{max}}$ was reasonably well predicted by environment ($r^2 = 0.64$, $P<0.001$). It was predominantly affected by the N level experienced by plants during growth (36% of explained variance), with a high N level leading to higher $I_{\text{max}}$ values. $I_{\text{max}}$ was then positively affected by PPFD (7%), $h_t$ (13%), and PPFD times $T_g$ (5%) and was negatively affected by soil moisture level (12%), $T_g$ (9%), and PPFD times $h_t$ (18%). $V_{C_{\max}}$, which was significantly predicted from environmental condition during growth ($r^2 = 0.66$, $P<0.001$), was mainly affected by $T_g$ (33%, negatively), N level (25%, positively) and soil moisture level (15%, negatively). Then, $V_{C_{\max}}$ was positively affected by PPFD (8%) and $k_3$ (5%) and was negatively affected by $CO_2$ level (5%) and PPFD times $h_t$ (8%).

$J_{\text{max}}$ was significantly predicted from environment ($r^2 = 0.51$, $P<0.001$) and the variance was shared between $CO_2$ level (27%, positively), $h_t$ (19%, positively), and PPFD times $h_t$ (24%, negatively). Note that $J_{\text{max}}$ increased with $CO_2$ concentration as reviewed by Ainsworth and Long [33]. The remaining variance was positively explained by PPFD (6%) and PPFD times $T_g$ (6%) and negatively explained by N and moisture levels (10 and 8%, respectively). $k_3$ was significantly predicted ($r^2 = 0.44$, $P<0.001$) and the variance was predominantly explained by N level (65%), with higher $k_3$ at lower N availability level, as also reviewed by Ainsworth and Long [33]. The temperature experienced by leaves during the preceding month was also an important driver of $k_3$ (25%), with lower $k_3$ at higher temperature. The remaining variance was positively explained by PPFD (2%) and $h_t$ (4%) and negatively explained by PPFD times $h_t$ (3%).

Once the multiple regression models were established for each leaf photosynthetic parameter, we tested by bootstrap analysis if their prediction was robust enough to satisfy the coordination hypothesis. All random datasets generated by bootstrap ($n = 220$) gave significant regression models (Tables S5–S6). The parameters values of these regression models were used with the remainder of the data ($n = 293–220 = 70$) to predict leaf photosynthetic parameters values. Photosynthetic parameters values were then used to predict $W_c$, $W_l$ and $N_{ac}$. We found that $W_c$ matched $W_c$ (Fig. 2A) and $N_{ac}$ matched $N_{ac}$ (Fig. 2B, RRMSE = 0.2), whatever the random dataset to which it was applied (Tables S5–S6).

In an attempt to falsify the leaf photosynthesis coordination hypothesis, we have randomized environmental growth conditions among observations. This randomization resulted in a strong mismatch between $W_c$ and $W_l$ (RRMSE = 0.76; slope = 0.60±0.33; $r^2 = 13%$) as well as between $N_{ac}$ and $N_{ac}$ (RRMSE = 0.72; slope = 0.80±0.40; $r^2 = 17%$).
Prediction from Our Leaf Photosynthesis Coordination Model

Under standard environmental conditions, $N_{\text{leaf}}$ varied significantly with $k_3$ and $f_{\text{max}}$ (Fig. 3A). $N_{\text{leaf}}$ decreased with increasing $k_3$ (Fig. 3A), which imposed a strong constraint on this physiological trait. For a given leaf $N_{\text{leaf}}$, high values of $k_3$ did not affect $A_n$ (Fig. 3B), but $PNUE$ increased linearly with $k_3$ (Fig. 3C). For a given $k_3$ value, both $N_{\text{leaf}}$ (Fig. 3A) and $A_n$ (Fig. 3B) displayed saturating responses to increasing $f_{\text{max}}$. As a consequence, $PNUE$ was little affected by $f_{\text{max}}$ (Fig. 3C). In our model (Eqn 1), $SLA$ and $f_{\text{max}}$ affected $N_{\text{leaf}}$, but did not affect $N_{\text{leaf}}$ and consequently $A_n$ and $PNUE$. Since $SLA$ displayed a higher degree of variation, the leaf structural content per unit area and consequently the leaf N content were strongly dependent on $SLA$. Thus, the leaf structural N content per unit area and the leaf N content followed an inverse relationship as $SLA$ increased.

When using overall dataset means of the leaf photosynthetic traits, $N_{\text{leaf}}$ varied significantly with radiation and temperature (Fig. 3D). $N_{\text{leaf}}$ increased linearly with $PPFD$ and decreased with $T_g$ according to a logistic curve (Fig. 3D, Fig. S2). For a given $N_{\text{leaf}}$, temperature affected $A_n$ according to a quadratic curve with an optimal $T_g$ around 20°C although $PPFD$ affected linearly $A_n$ (Fig. 3E). As a consequence, $PNUE$ was affected by $T_g$ according to a peak curve with an optimal $T_g$ at 25°C and was positively affected by $PPFD$ according to a logarithmic curve (Fig. 3F).

Discussion

A Successful Test of the Coordination Hypothesis of Leaf Photosynthesis

The coordination hypothesis provides a testable analytical solution to predict both photosynthetic capacity and area-based leaf N content and, hence, to couple photosynthetic C gain and leaf N investment. With the large dataset used in this study, we could not falsify this testable hypothesis. Therefore, our results strongly support the validity of the leaf photosynthetic coordination hypothesis across a wide range of C$_3$ plant species and of environmental conditions.

Our coordination model linking leaf photosynthesis, stomata conductance and nitrogen investment has a total of 33 parameters. Only four parameters are directly related to a coordinated investment of leaf N into carboxylation capacity ($V_{\text{Cmax}}$; RuBP carboxylation; Rubisco) and RuBP regeneration ($J_{\text{max}}$, RuBP regeneration; light harvesting): $J_{\text{max}}$, the ratio of $J_{\text{max}}$ to $V_{\text{Cmax}}$ determines the photosynthetic capacity; and $k_3$, the ratio of $V_{\text{Cmax}}$ to leaf photosynthetic N content ($N_{\text{leaf}}$) determines the fraction of metabolic leaf N invested in photosynthesis. The ratio of $f_{\text{max}}$ to $SLA$ determines the fraction of non-metabolic N per unit total leaf N.

Photosynthetic parameter values vary to a considerable extent across species and environmental conditions in agreement with previous studies [2,3,39]. For instance, Wullschleger [2] reported that, when expressed at a reference temperature of 20°C, $V_{\text{Cmax}}$ varies in the range 5–142 (µmol m$^{-2}$ s$^{-1}$); $J_{\text{max}}$ in the range 11–251 (µmol m$^{-2}$ s$^{-1}$) and $T_{\text{air}}$, in the range 0.9–3.8 (dimensionless). Despite similar large differences in our dataset in parameter values across species and environmental conditions, our photosynthetic coordination model accounts for 93% of the total variance in $N_{\text{n}}$. Moreover, the model has a low systematic RRMSE with no systematic bias. The statistical validity of this model supports the conclusion that sunlight mature leaves of C$_3$ plants tend to achieve photosynthetic coordination in a wide range of both optimal and sub-optimal environmental conditions.

Along the vertical profile of C$_3$ plant canopies, an empirical scaling law between area based leaf N content and transmitted $PPFD$ has often been reported [15,17,40,41] and has been determined as the predominant factor of N decline relative to others like leaf age or N demand [12,40,41]. Various hypotheses have been put forward to explain this observation [11,22,42,43]. Our model of the coordination hypothesis matches this scaling law, since $N_{\text{leaf}}$ scales with radiation ($PPFD$) along the vertical canopy profile (Eqn 2). Air temperature ($T_{\text{air}}$), relative air humidity ($h_r$) and ambient CO$_2$ concentration ($C_{\text{a}}$) also vary with depth within the canopy. At a given $PPFD$, higher $h_r$ and lower $T_{\text{air}}$ at

Figure 2. Tests of the coordination hypothesis using values of leaf photosynthetic traits predicted from environmental growth conditions. A) Relationship between the predicted rates of RuBP carboxylation/oxygenation ($W_c$) and RuBP regeneration ($W_i$) under plant growth conditions. B) Relationship between predicted ($N_{\text{leaf}}$) and observed ($N_{\text{leaf}}$) leaf N content. The insert in Fig. 2B shows the same relationship without the very high observed $N_{\text{leaf}}$ values for the PFT1. Symbols are as for Fig. 1. doi:10.1371/journal.pone.0038345.g002
Figure 3. Relationships between simulated photosynthetic leaf N content ($N_{p_{ac}}$) (A), net photosynthesis ($A_n$) (B) and photosynthetic N use efficiency (PNUE) (C) and the photosynthetic traits $k_3$ and $J_{fac}$ under standard mean environmental conditions (PPFD).
= 666 μmol m⁻² s⁻¹, Tₑ = 16.9°C, hₑ = 0.74). kₛ is the ratio between Vₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ euler evel m student, m student (Fig. 3D–F). These results are in accordance with the prediction by doi:10.1371/journal.pone.0038345.g003

Senescence [47]. Structural N would therefore not be redistributed in the same range as those found for dead leaves after N resorption at 15–50% of Nₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ euler evel m student, m student, Nₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ euler evel m student, euler evel m student, m student for sclerophyllous tree, shrub and vine species, [34,44]) and in a study provides a means for disentangling: i) the direct environment to plant growth environment. Averages over the dataset of leaf photosynthetic parameters (kₛ, Jₑₑₑₑₑₑ euler evel m student, euler evel m student) are used (kₛ = 59.1 μmol g⁻¹ N₂ s⁻¹, Jₑₑₑₑₑ euler evel m student = 2.45, SLA = 17.7 m² kg⁻¹ DM). The mesh for temperature is 0.5°C between 10 and 30°C and the mesh for radiation is 50 μmol m⁻² s⁻¹ between 300 and 1200 μmol m⁻² s⁻¹. The values of hₑₑₑₑₑₑₑₑₑₑₑₑₑₑ euler evel m student and Tₑₑₑₑₑₑₑₑ euler evel m student were fixed at 0.8 and 20°C, respectively. Aₑₑₑₑₑₑ euler evel m student was calculated with the coordinated leaf protein content and PNEU was calculated as the ratio between Aₑₑ euler evel m student and Nₑₑₑₑₑₑ euler evel m student.

doi:10.1371/journal.pone.0038345.g003

Determinism of Leaf N Content Variation

Genetic and environmental factors have long been recognized to interact in determining the Aₑₑₑₑₑₑ euler evel m student vs. leaf N relationship [5]. Our study provides a means for disentangling: i) the direct environmental effects on leaf photosynthetic N content (Nₑₑₑₑₑₑ euler evel m student); ii) the role of photosynthetic parameters for Nₑₑₑₑₑₑ euler evel m student in a given environment; and iii) the response of photosynthetic parameters i.e. the plant acclimation to plant growth environment.

First, for a given set of plant parameters, positive effects of radiation and negative effects of air temperature, air relative humidity and CO₂ concentration on Nₑₑₑₑₑₑ euler evel m student are predicted by Eqn 2 (Fig. 3D–F). These results are in accordance with the prediction by Farquhar et al’s canopy photosynthesis model [49], which links stomatal control with leaf area and leaf N content by optimizing both water and nitrogen use efficiency and predicts an increase of leaf N content and Vₑₑₑₑₑₑₑₑₑₑₑ euler evel m student with mean radiation increase [24,50] and mean annual rainfall [49,51]. According to the hypothesis, changes in Nₑₑₑₑ euler evel m student affect both biochemical photosynthesis capacities, Vₑₑₑₑₑₑ euler evel m student and Jₑₑₑₑₑₑ euler evel m student. Indeed, seasonal variations in Vₑₑₑₑₑₑ euler evel m student and Jₑₑₑₑₑₑ euler evel m student have been observed for a number of plant species [52,53] and were related to changes in Rubisco and cytochrome-f contents in Polygonum cuspidatum [54]. Including photosynthetic capacity (Vₑₑₑₑₑₑ euler evel m student and Aₑₑₑₑₑ euler evel m student) and its relationship to leaf N content in terrestrial biosphere models resulted in substantial changes in gross primary productivity with latitude [7]. Coupled environmental variations in PPFD, Tₑₑₑ euler evel m student, hₑ euler evel m student and Cₑ euler evel m student simultaneously affect Nₑ euler evel m student throughout time, which has major implications for gross primary productivity and PNEU of a given species or genotype.

Second, the coordination hypothesis implies that under a given environment, Nₑ euler evel m student tends toward a unique coordinated Nₑ euler evel m student value (Eqn 2). As shown by the analysis of model sensitivity to parameters and input variables (Text S1, Fig. S3), kₛ and Jₑ euler evel m student are among the most important determinants of Nₑ euler evel m student value. Assuming a single average value of kₛ and of Jₑ euler evel m student for all species in the dataset would increase Nₑ euler evel m student RRMSE by 50% (Table 3A). However, using a single Jₑ euler evel m student value by PFT with species-specific kₛ and SLA values provided a strong accuracy for Nₑ euler evel m student prediction. This result is consistent with the strong linear relationship between Vₑ euler evel m student and Jₑ euler evel m student reported by Wullschleger [2] among 109 species, which probably indicates a phylogenetic constraint for Jₑ euler evel m student. Under given environmental conditions, our results show that there is no single combination of kₛ and Jₑ euler evel m student that can maximize both Aₑ euler evel m student and PNEU (Fig. 3A-C). Therefore, variable combinations of these photosynthetic traits could be equally relevant. This relative independency of kₛ and Jₑ euler evel m student suggests that these functional traits (ensa [55]) correspond to possibly overlooked axes of differentiation among C₃ plant species. kₛ, which modulates the N investment at a given Aₑ euler evel m student, could be related to a plant strategy of nutrients conservation [56], Jₑ euler evel m student, which increases Aₑ euler evel m student for a given kₛ, could be related to a plant strategy of nutrients exploitation. However, the lack of correlation between these two photosynthetic traits and SLA, which is a key morphological trait separating exploitative and conservative species strategies for nutrient use [56], suggests that these physiological traits form a secondary axis of differentiation across C₃ species.

Third, some environmental growth conditions such as PPFD, Tₑ euler evel m student, hₑ euler evel m student and N availability had significant effects on kₛ and Jₑ euler evel m student. The increase in kₛ at low N availability tends to reduce Nₑ euler evel m student and, hence, N demand for leaf construction thereby increasing PNEU. The increase in kₛ with PPFD tends to compensate for the direct positive effect of PPFD on Nₑ euler evel m student, thereby lowering N demand for leaf construction under high light environments. Similarly, the decrease of kₛ with Tₑ euler evel m student mitigates the direct negative effect of temperature on Nₑ euler evel m student, thereby equalizing the N demand for a range of temperature. Mostly independently from changes in kₛ (since these two traits are not correlated across plant species), Jₑ euler evel m student increases with Cₑ euler evel m student in agreement with the lower decline under elevated CO₂ of Jₑ euler evel m student compared to Vₑ euler evel m student [39]. Moreover, Jₑ euler evel m student is negatively related to PPFD, which is in good agreement with the higher allocation of leaf N to chlorophyll observed in low PPFD acclimation experiments [57]. Like the increase in kₛ, the decrease in Jₑ euler evel m student with PPFD tends to compensate for the direct positive effect of PPFD on Nₑ euler evel m student, especially for species with low kₛ value. Finally, the effect of temperature on Jₑ euler evel m student is not significant which is in agreement with previous studies that reports constant Jₑ euler evel m student with temperature (e.g. [33]).

Uncertainties in the Calculation of the Coordinated Leaf Photosynthetic N Content

Our model takes into account the two main biochemical processes controlling leaf photosynthesis as well as the biophysical
process controlling stomatal conductance. Recently, leaf mesophyll conductance has also been identified as an important biophysical limitation of photosynthesis [30–60], particularly for species with low SLA by decreasing $V_{\text{C}_{\text{max}}}$ more than $J_{\text{max}}$ [61,62] and particularly during plant acclimation to water stress condition [50,59]. Applying mesophyll conductance in our model would first require recalculating $V_{\text{C}_{\text{max}}}$ parameter from a non-rectangular hyperbola of the $A_{\text{c}}-C_{\text{i}}$ curve and with a new set of Rubisco kinetic constants, for example [58]. Moreover, it would also require the incorporation in our model of the CO$_2$ diffusion mechanism between intercellular and chloroplast spaces according to a mesophyll conductance parameter [59,60]. Furthermore, the coupling between $A_{\text{c}}$ and $g_s$ leading to the calculation of $A_{\text{c}}$ would require solving a new system of equations and unknowns. Finally, this would require additional mesophyll conductance data, which were not available in our dataset. The inclusion of a variable mesophyll conductance [61,62], as well as of other mechanisms implied in plant responses to water deficits [63], would allow testing the photosynthetic coordination hypothesis under severe abiotic stress conditions. With the coordination model reported here that does not include these processes, $N_v$ values are lower than $N_a$ values under more severe abiotic stress conditions (data not shown).

The calculation of $N_{\text{fac}}$ relies on a number of plant parameter and environmental variables, leading to further uncertainties (see Text S1, Table S2 and Fig. S2–S3 for full details). Apart from SLA, $k_3$ and $J_{\text{fac}}$, all plant parameters were assumed to have a single set of values across the entire dataset (Table 2). Since the photosynthetic model was shown to be little sensitive to most of these parameters (Text S1, Fig. S3), using species-specific values would only marginally increase the accuracy of $N_a$ prediction.

Implications

Overall, our study confirms the basic assumption of the coordination hypothesis: leaves coordinate the development of $V_{\text{C}_{\text{max}}}$ and $J_{\text{max}}$ such that $W'_c$ equals $W'_j$. This opens opportunities to couple C and N at a global scale by incorporating the coordination hypothesis into dynamic global vegetation models (DGVMs). However, the applicability of this hypothesis for improved prediction of photosynthetic capacity and leaf nitrogen content depends on the accuracy at which we can determine key parameters of the combined photosynthesis - stomatal conductance – leaf N model as well as the timescale of plant regulatory photosynthesis mechanisms. The two key parameters $J_{\text{fac}}$ and $k_3$ seem to be predictable from a combination of environmental growth conditions - probably due to the strong dependence of the development of the photosynthetic machinery on environment variables – and information about plant growth form or PFT. However, the morphological trait SLA does not seem to be predictable with sufficient accuracy from environmental conditions which is consistent with the large functional diversity found in a given environment [64]. SLA needs to be defined at least by PFT and preferably by species. This study thus confirms the relevance of leaf morphology, represented by SLA, in photosynthesis, which has been pointed out before, e.g. [56]. However, SLA is one of the best-studied plant traits worldwide (e.g. [36]) and it may be possible to determine SLA with sufficient accuracy for a large range of C$_3$ species. Finally, although the turnover of photosynthetic enzymes like Rubisco can be seen as very constrained within the C$_3$ plant kingdom, to our knowledge there is no study that investigates its variability across species. We therefore stress the need for further comparative research quantifying the variability of photosynthetic enzyme turnover across C$_3$ species. Further tests of the coordination hypothesis will require, during plant growth, coupled measurements of microclimate, of leaf gas exchanges and of photosynthetic traits, including the dynamics of Rubisco, within the canopy [65].

Conclusion

This study bridges a gap concerning the coupling of C and N fluxes in C$_3$ plant species. It confirms the basic assumption of the leaf photosynthesis coordination hypothesis and demonstrates that this hypothesis can be successfully applied across species and PFTs and under a wide range of climates. Moreover, we have shown that $k_3$ and $J_{\text{fac}}$ in combination with SLA are major plant functional traits, which reflect plant adaptation to light, temperature and N availability during growth. Surprisingly, few studies provide both leaf photosynthetic parameters and environmental conditions during plant growth. Improved datasets combining the $k_3$ and $J_{\text{fac}}$ photosynthetic traits with the SLA morphological trait are needed to further increase our understanding of leaf economics (C–N stoichiometry) and plant strategies. The leaf photosynthesis coordination model reported here has been successfully used in a patch scale grassland vegetation model [66,67]. Further applications include modeling at regional and global scales the role of plant diversity for the carbon and nitrogen cycles.

Supporting Information

Figure S1 Details on the leaf photosynthesis coordination hypothesis. Variation of leaf carboxylation rates with leaf nitrogen content for three levels of radiations (A–C). According to the leaf photosynthesis coordination theory, a leaf photosynthetic N content is determined as colimiting the carboxylation/oxygenation of ribulose-1,5-bisphosphate (RuBP) by the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; $W'_c$), and the regeneration of RuBP by the electron transport chain ($W'_j$). Below $N_{\text{fac}}$, the photosynthesis will be limited by the Rubisco activity and therefore by the amount of leaf proteins. Beyond $N_{\text{fac}}$, the marginal gain of photosynthesis per unit of leaf proteins is weak. Along the vertical canopy profile, $N_{\text{fac}}$ declines with transmitted radiation when all other variables are equal.

(TIF)

Figure S2 Mean temperature functions of the maximum rates of carboxylation ($V_{\text{C}_{\text{max}}}$) and electron transport ($J_{\text{max}}$) and their ratio ($\Phi_{\text{fac}}/\Phi_{V_{\text{C}_{\text{max}}}}$). Functions were calculated using the parameters related to temperature sensitivity (activation and deactivation enthalpies and entropy) as calibrated by Katge & Knorr (2007) for many species (48 species for $V_{\text{C}_{\text{max}}}$, 32 for $J_{\text{max}}$ and 29 for their ratio). The error bars correspond to the standard errors among species representing the inter-specific variability.

(TIF)

Figure S3 Sensitivity analysis of the photosynthesis-stomatal conductance model. Following Felix & Xanthoulis (2005), a sensitivity analysis of the models calibrated for Dactylis glomerata with common one-to-one variation of parameters (±15%). Output variables are shown as lines, parameters as columns. The sensitivity index (IOS) was calculated as the maximal ratio of output variation to parameter variation during a climatic scenario (air temperature, PPFD, $k_3$ and $C_3$) recorded from an upland site in central France (Theix, 45°43’N, 03°01’E, 870 m) for years 2003–2004. Color tones indicate sensitivity index (positive, red; negative, blue).

(TIF)

Table S1 Dataset used for the validation of leaf photosynthesis coordination. The excel file includes the leaf
photosynthetic and the environmental growth conditions used to calculate $W_c$, $W_j$ and $N_{ac}$. (XLS)

**Table S2** Range of the observed values among literature of the parameters used in the leaf photosynthesis – stomatal conductance model. The categories were the minimum, the maximum, the median and the percentage of variation of parameters range. The sources of observations were also reported. The sources, where the minimum and maximum values were observed, were annotated with – and +. A reference temperature of 20°C was used. (DOC)

**Table S3** Multiple regression analyses of $V_{c\text{max}}$ and $J_{\text{max}}$ from environmental growth conditions for the bootstrap analysis. Independent variables: X1: air CO2 concentration ($C_a$); X2: N level; X3: soil H2O level; X4: radiation (PPFD); X5: air relative humidity ($h_a$). The number of observations was 236. (DOC)

**Table S4** Multiple regression analyses of $k_3$ and $J_{\text{fac}}$ from environmental growth conditions for a bootstrap analysis. Independent variables were the same as Table S3. The number of observations was 236. (DOC)

**Table S5** Prediction of $W_c$ and $W_j$ (µmol m$^{-2}$ s$^{-1}$) in using the parameters $V_{c\text{max}}$ and $J_{\text{max}}$ calculated from regression analyses on the independent part of the dataset in a bootstrap analysis (Table S3). Characteristics of the $W_c/W_j$ relationship. The intercepts of regression for each PFT were set to zero (since there were not significantly different from zero) to estimate the slopes. RRMSE: relative root mean square error. (DOC)

**Table S6** Prediction of $N_{ac}$ in using the parameters $k_3$ and $J_{\text{fac}}$ calculated from the regression analyses on the independent part of the dataset in a bootstrap analysis (Table S4). Characteristics of the relationship between predicted and observed leaf N content ($N_{ac}/N_a$, gN m$^{-2}$). The intercepts of regression for each PFT were set to zero (since there were not significantly different from zero) to estimate the slopes. Abbreviation: RRMSEs and RRMSEU are systematic and unsystematic relative root mean square error, respectively. (DOC)

**Table S7** Dependence of leaf photosynthetic parameters on plant functional type (PFT). ANOVA model and mean comparison test by LSD method of the PFT effect on leaf photosynthetic traits used in the test of coordination hypothesis ($V_{c\text{max}}$, $J_{\text{max}}$, $k_3$, $J_{\text{fac}}$ and SLA). The values of $k_3$ and $J_{\text{fac}}$ were log-transformed and all residuals followed a normal distribution. For a given variable, PFTs with the same letter belong to the same group. (DOC)

**Text S1** Sensitivity analysis of the photosynthesis – stomatal conductance model. (DOC)

**Text S2** Demonstration of the formalism of the coordinated leaf photosynthetic N content. (DOC)

**Acknowledgments**

Authors thank V. Allard, N. Gross, J. Schymanski, N. Viovy and P. Giais for constructive comments on a previous version of the manuscript.

**Author Contributions**

Conceived and designed the experiments; JFS VM. Analyzed the data; VM PM JK JFS. Wrote the paper: VM PM JK JFS. Assembled the data: JK VM PM FG GE. Provided model development and statistical methods: VM. Commented on the manuscript: GE SF FG.

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