Tansley reviews

A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types

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Summary

Extensive within-canopy light gradients importantly affect the photosynthetic productivity of leaves in different canopy positions and lead to light-dependent increases in foliage photosynthetic capacity per area ($A_A$). However, the controls on $A_A$ variations by changes in underlying traits are poorly known. We constructed an unprecedented worldwide database including 831 within-canopy gradients with standardized light estimates for 304 species belonging to major vascular plant functional types, and analyzed within-canopy variations in 12 key foliage structural, chemical and physiological traits by quantitative separation of the contributions of different traits to photosynthetic acclimation. Although the light-dependent increase in $A_A$ is surprisingly similar in different plant functional types, they differ fundamentally in the share of the controls on $A_A$ by constituent traits. Species with high rates of canopy development and leaf turnover, exhibiting highly dynamic light environments, actively change $A_A$ by nitrogen reallocation among and partitioning within leaves. By contrast, species with slow leaf turnover exhibit a passive $A_A$ acclimation response, primarily determined by the acclimation of leaf structure to growth light. This review emphasizes that different combinations of traits are responsible for within-canopy photosynthetic acclimation in different plant functional types, and solves an old enigma of the role of mass- vs area-based traits in vegetation acclimation.

I. Introduction

Variation in daily integrated quantum flux density is the most striking feature of plant canopies. In dense canopies, the gradient in light availability from canopy top to bottom can be > 50-fold (Hirose et al., 1988; Koike et al., 2001; Valladares, 2003; Niinemets & Anten, 2009), whereas relatively open canopies exhibit light gradients as large as 10–20-fold (Hirose et al., 1988;
Werger & Hirose, 1988; Rambal, 2001; Joffre et al., 2007). Even in free-standing plants, the top foliage shades the leaves below and in the interior of the canopy, leading to strong light gradients (e.g. Le Roux et al., 1999). As a key acclimation response, foliage photosynthetic capacity (the light-saturated net assimilation rate per unit area, \( A_s \)) increases with increasing long-term light availability within the canopy. Thus, leaves in different canopy positions with different physiological potentials are fine-tuned to their past and contemporary light conditions (Hirose & Werger, 1987a; Ellsworth & Reich, 1993; Anten, 1997; Pons & Anten, 2004; Niinemets & Anten, 2009). As a result of \( A_s \) acclimation, whole-canopy integrated carbon gain is greater than if \( A_s \) were constant throughout the canopy (Gutschick & Wiegel, 1988; Baldocchi & Harley, 1995; Lloyd et al., 2010; Dewar et al., 2012). Although the importance of the consideration of within-canopy acclimation of \( A_s \) in the modeling of vegetation carbon gain has been well recognized at single plant, ecosystem and landscape scales (e.g. Baldocchi & Harley, 1995; Cescatti & Niinemets, 2004; Anten, 2005; Lloyd et al., 2010), recent world-scale modeling efforts have further demonstrated that the incorporation of within-canopy \( A_s \) gradients can even result in major improvements to world-scale carbon gain estimations by the Earth System models (Bonan et al., 2012). Thus, a detailed understanding of within-canopy photosynthetic acclimation has broad implications for the prediction of vegetation productivity through organismal to global scales.

In understanding the within-canopy acclimation responses of \( A_s \), it is relevant to consider that a wide range of leaf structural and chemical traits vary between canopy top and bottom, including leaf dry mass per unit area, leaf nitrogen content and nitrogen partitioning among proteins of the photosynthetic machinery (Hirose & Werger, 1987a; Ellsworth & Reich, 1993; Anten, 1997; Pons & Anten, 2004; Niinemets & Anten, 2009). Within-canopy changes in these key functional traits ultimately determine the within-canopy photosynthetic modifications, but there is currently limited understanding of the overall degree of within-canopy plastic modification in \( A_s \), of the extent to which different traits control photosynthetic acclimation and how the controlling traits differ among plant functional types.

In this review, we first define the functional relationships between the key traits underlying the within-canopy variation in foliage photosynthetic potentials. We then describe the construction of a worldwide database on within-canopy trait variation and the derivation of standardized light availability estimates for all light gradients in the database. Because light is one of the most variable environmental drivers, we deem that standardization of light availability across the studies is absolutely essential for any meta-analysis intending to gain an insight into the light effects on plant functioning. We further use this database to cast light on structural, chemical and allocational controls on \( A_s \), and demonstrate a huge variation among traits in their overall light-dependent variability and in their control on \( A_s \). This review highlights that variations in trait plasticity, and in their relative controls on \( A_s \), are strongly dependent on plant functional type. In particular, species in the fast return end of the leaf economic spectrum (Wright et al., 2004; Shipley et al., 2006), with high rates of leaf turnover and canopy development, are characterized by modifications in \( A_s \) as a result of nitrogen reallocation among leaves and changes in nitrogen partitioning within leaves. However, within-canopy acclimation in species in the slow return end of the spectrum, with low rates of leaf turnover and canopy development, is primarily driven by the acclimation of leaf structure to the light environment during leaf growth. Thus, we contend that foliage photosynthetic acclimation to within-canopy light gradients is fundamentally different among plant functional types.

There is a debate over which leaf traits provide informative insight into leaf functioning with emphasis on the usefulness of area- vs mass-based expressions of leaf photosynthetic capacity (Lloyd et al., 2013; Westoby et al., 2013; Poorter et al., 2014). We address this debate by arguing that, in order to understand within-canopy photosynthetic acclimation, we must distinguish between the effects of leaf structure, nitrogen content and partitioning on foliage biochemical potentials, and therefore, consider the variations in both mass- and area-based leaf traits.

### II. Defining the structural, chemical and partitioning controls on foliage photosynthetic potentials

Modifications in leaf structural, chemical and allocational traits are directly related to the maximum potential photosynthetic capacity of leaves per unit leaf area, \( A_s (\mu\text{mol m}^{-2} \text{s}^{-1}) \). In this section, we review established relationships between different leaf traits and \( A_s \). To separate the effects of modifications in the photosynthetic capacity of single leaf cells, and the number of cells per unit leaf area, \( A_s \) can be expressed as the product of the photosynthetic capacity per unit leaf dry mass, \( A_M (\mu\text{mol g}^{-1} \text{s}^{-1}) \), and leaf dry mass per unit area, \( M_A (\text{g m}^{-2}) \):

\[
A_s = A_M M_A. \quad \text{Eqn 1}
\]

Dry mass per unit area is the key integrated trait characterizing the biomass cost of formation of a unit of leaf area (Poorter et al., 2009) and, as such, is strongly driven by the availability of carbon for the construction of photosynthetic machinery (Gutschick & Wiegel, 1988; Kull & Krujit, 1999). The photosynthetic capacity of single leaf cells, in turn, depends on both the cellular nitrogen content and the nitrogen investment in photosynthetic machinery, which is commonly characterized by the photosynthetic nitrogen use efficiency (\( E_N (\mu\text{mol (g N)}^{-1} \text{s}^{-1}) \)), that is, the rate of photosynthesis per unit leaf nitrogen content (Hirose & Werger, 1987b, 1994; Hikosaka et al., 1998; Hirose & Bazzaz, 1998; Yasumura et al., 2002; Escudero & Mediavilla, 2003; Pons & Westbeek, 2004):

\[
A_M = E_N N_M. \quad \text{Eqn 2}
\]

where \( N_M (\text{g g}^{-1}) \) is the leaf nitrogen content per leaf dry mass. Converting to a leaf area basis, and considering that leaf nitrogen content per leaf area, \( N_A (\text{g m}^{-2}) \), is:
\[ N_A = N_M M_A \quad \text{Eqn 3} \]

we obtain:

\[ A_A = E_N N_M M_A = E_N N_A. \quad \text{Eqn 4} \]

\( E_N \) is derived from measurements of photosynthetic rates and nitrogen content, but this does not mean that \( E_N \) is necessarily dependent on or correlated with photosynthetic rate and/or nitrogen content expressed per unit area or mass. Indeed, classical studies demonstrate that \( E_N \) can vary widely at a given rate of photosynthesis and a given leaf nitrogen content (Field & Mooney, 1986; Evans, 1989). Although correlations among the traits defined by Eqns 2–4 have been reported (Wright et al., 2004; Westoby et al., 2013), any such correlation would not invalidate the functional dependences of photosynthetic capacity on the stacking of photosynthetic cells per unit leaf area, cellular nitrogen content and/or the allocation of cellular nitrogen to photosynthetic proteins.

A key disadvantage with the concept of \( A_A \) is that, at current ambient \( \text{CO}_2 \) concentrations, it can vary with changes in stomatal diffusion conductance to \( \text{CO}_2 \) (\( g_{\text{CO}_2} \)). This is important as the limitation of photosynthesis by \( g_{\text{CO}_2} \) can vary through the canopy, especially during stress conditions (e.g. Aasamaa et al., 2004; Niinemets et al., 2004b). In addition, photosynthetic nitrogen use efficiency does not provide a direct measure of nitrogen investment in rate-limiting components of photosynthesis. These limitations of the use of \( A_A \) can be elegantly addressed using the \( C_3 \) plant biochemical photosynthesis model of Farquhar et al. (1980).

In particular, two key model parameters, the maximum carboxylase activity of ribulose-1,5-bisphosphate (Rub\( \Pi \)) carboxylase/oxygenase (Rubisco) (\( V_{\text{cmax}} \)) and the capacity for photosynthetic electron transport rate (\( J_{\text{max}} \)), allow for alterations in foliage biochemical photosynthesis potentials to be analyzed directly without interfering stomatal controls. \( V_{\text{cmax}} \) characterizes the capacity of the dark reactions of photosynthesis (\( \text{CO}_2 \) fixation) and \( J_{\text{max}} \) the capacity of the light reactions of photosynthesis (Rub\( \Pi \) regeneration by ATP and NADPH produced in the photosynthetic electron transport chain in light). In practice, both light and dark reactions take place in light, and light-saturated photosynthesis is driven by both \( V_{\text{cmax}} \) and \( J_{\text{max}} \). Occasionally, a third limitation, the capacity for triose phosphate utilization (Sharkey, 1985), can constrain photosynthesis, but there are few data on within-canopy variation in this trait (for an exception, see Urban et al., 2003).

Analogous to \( A_A \), \( V_{\text{cmax}} \) and \( J_{\text{max}} \) per unit area (\( V_{\text{cmax}, A} \) and \( J_{\text{max}, A} \), both in \( \text{mmol m}^{-2} \text{s}^{-1} \)) can be expressed as composites of independent variables. As Eqn 1 states, both the area-based potentials can be expressed as the products of \( M_A \) and mass-based potentials \( V_{\text{cmax}, M} \) and \( J_{\text{max}, M} \) (both in \( \text{mmol g}^{-1} \text{s}^{-1} \)). \( V_{\text{cmax}, M} \) can be further expressed as:

\[ V_{\text{cmax}, M} = 6.25 V_{\text{c}} \kappa F_R N_M M_A, \quad \text{Eqn 5} \]

where \( V_{\text{c}} \) is the specific activity of Rubisco, that is, the maximum rate of Rub\( \Pi \) carboxylation per unit Rubisco protein (\( \text{mmol g}^{-1} \text{s}^{-1} \)), \( \kappa \) is the fraction of active Rubisco sites, \( F_R \) is the fraction of leaf nitrogen in Rubisco, \( N_M \) is the leaf nitrogen content per dry mass (\( \text{g g}^{-1} \)) and 6.25 (\( \text{g g}^{-1} \)) is the nitrogen content of Rubisco protein (Niinemets & Tenhunen, 1997). The activation state of Rubisco, \( \kappa \), can vary as a function of environmental characteristics (e.g. Ethier et al., 2006; Yamori et al., 2006), although \( V_{\text{cmax}} \) in studies exploring functional leaf trait relationships is characteristically estimated in non-stressed plants with maximum activation state of Rubisco. \( J_{\text{max}, M} \) is further given as:

\[ J_{\text{max}, M} = 8.06 J_{\text{mc}} F_R N_M, \quad \text{Eqn 6} \]

where \( J_{\text{mc}} \) is the capacity for photosynthetic electron transport per unit cytochrome \( f \). \( F_R \) is the fraction of nitrogen in rate-limiting proteins of photosynthetic electron transport and the factor 8.06 considers the nitrogen content of rate-limiting proteins and the molar stoichiometry relative to cytochrome \( f \) (Niinemets & Tenhunen, 1997). The use of cytochrome \( f \) as the key limiting step assumes that the capacity for linear electron transport rate is determined by the amount and turnover rate of electron carriers between photosystems I and II (for a discussion, see Evans, 1988; Evans & Seemann, 1989; Niinemets & Tenhunen, 1997).

On an area basis, \( V_{\text{cmax}, A} \) is further expressed as:

\[ V_{\text{cmax}, A} = 6.25 V_{\text{c}} \kappa F_R N_M M_A = 6.25 V_{\text{c}} \kappa F_R N_A, \quad \text{Eqn 7} \]

and \( J_{\text{max}, A} \) is given analogously.

The specific activities, \( V_{\text{c}} \) and \( J_{\text{mc}} \), have been widely considered to be conserved for \( C_3 \) plants (e.g. Niinemets & Tenhunen, 1997; Le Roux et al., 2001; Walcroft et al., 2002; Urban et al., 2003; Rogers, 2014). Although there is evidence of a certain interspecific variability in \( V_{\text{c}} \) (e.g. Galmés et al., 2014a,b), \( V_{\text{c}} \) and \( J_{\text{mc}} \) can be considered to be invariable through the canopy of any given species. In principle, the Rubisco activation state could vary through the canopy, but to our knowledge, such a variation has not been studied so far. Considering \( V_{\text{c}} \) and \( J_{\text{mc}} \) as constants and assuming that \( \kappa = 1 \) (Rubisco is fully active), \( F_R \) and \( F_R \) are necessarily the apparent estimates of leaf nitrogen investment in rate-limiting components of photosynthetic machinery. In the absence of independent estimates of protein content in the rate-limiting components of photosynthetic machinery, \( F_R \) and \( F_R \) need to be estimated from the measurements of \( J_{\text{max}, M} \) and nitrogen content, but this does not mean that the estimated nitrogen allocation is autocorrelated with the source variables (Niinemets et al., 1998; Evans & Poorter, 2001; Le Roux et al., 2001; Walcroft et al., 2002).

With the outlined assumptions, the functional Eqns 1–7 define altogether 12 key structural, chemical, allocational and photosynthetic traits that make it possible to separate the sources of variation in leaf photosynthetic capacity (six variables, Eqns 1–4) and biochemical photosynthesis potentials (nine variables, Eqns 5–7). The approach based on the model by Farquhar et al. (1980) is more mechanistic, but there are far fewer data available on within-canopy variation in foliage biochemical photosynthesis potentials (Table 1). Nevertheless, the approaches are broadly analogous, sharing structural (\( M_A \)), nitrogen (\( N_M \), \( N_A \)) and allocation controls (\( E_N \) vs \( F_R \) and \( F_R \)). All of the 12 defined traits can vary through plant canopies, but to our knowledge, there has been no comprehensive
study analyzing the overall variation in these traits through plant canopies, examining how these variations differ among plant functional types, or quantitatively evaluating the extent to which the variations in the constituent variables ultimately contribute to within-canopy variations in leaf photosynthetic capacity.

### III. Construction of a global database on within-canopy variation in leaf structural, chemical and photosynthetic characteristics

#### 1. Data sources, gradient types, units and species classification

An extensive literature survey was carried out to find published data on within-canopy gradients in leaf morphology, chemistry and leaf photosynthetic properties. Only studies reporting within-canopy variation in at least three different canopy positions and with explicit measurements of within-canopy light conditions or cumulative leaf area index were considered. The survey identified 292 studies providing altogether 831 light gradients for 304 taxa belonging to different plant functional types (Supporting Information Table S1). The data on leaf functional traits were extracted from the studies, together with key meta-information. The meta-information included in the database was the plant growth environment (artificial vs field), the location of the study, year and time of sampling, the age of the sampled foliage, plant size, details of light measurements and, whenever pertinent, experimental treatment and the start of the treatment and/or planting date. For compound-leaved species, it was also noted whether the data referred to leaflets or entire leaves.

Several studies reported multiple light gradients in plant stands subject to various experimental treatments, including density and drought treatments, defoliation, thinning, pruning, extra illumination, shading, fertilization and atmospheric [CO₂] elevation.
(Table S1). In addition, replicate light gradients reported in the
same studies included different sites, multiple leaf age classes and
plant ages and sizes (Table S1). Replicates in time were often also
associated with differences in plant size (e.g. DeJong et al., 1989;
Vapaavuori & Vuorinen, 1989; Vapaavuori et al., 1989; Rosati et al.,
2000; Palmroth et al., 2002; Han et al., 2006; Han & Chiba,
2009), warranting a consideration of these gradients separately.
However, to reduce the effects of extreme treatments, rigorous
routines were developed for the inclusion of gradients from studies
including treatments (Table S1). On the other hand, individual
light gradients consisted of different-sized or aged individuals in
some studies, whereas other studies pooled replicates in time or
different leaf ages. In addition, data ranges for individual replicate
gradients in some multiple-gradient studies were too narrow and
the consideration of single gradients was impractical. Various
treatments applied in the original studies, the presence of replicate
gradients and pooled treatments are explicitly stated in Table S1.

For all leaf-level traits, the units were harmonized, and all data
were expressed per unit projected leaf area, as explained in
Notes S1. The species included in the database spanned a wide
range of vascular plant functional types (Table S1). Although a
large number of species were included in the database, some plant
functional types were only represented by a few species, and the
number of species available for different traits also varied. For
the purposes of statistical analyses, plant species were classified among
the functional types to include normally at least 20 light gradients
for each functional type (Table S1). Thus, functionally convergent
or related plant functional types with a limited number of species
were merged with more species-rich plant functional types as
explained in Table S1. The functional type classification was also
varied depending on the availability of observations for any given
trait. Finer scale plant functional types, such as annual and
perennial forbs and grasses, and C4 and C3 grasses for herbaceous
species and woody evergreens, separated according to climatic
origin (Mediterranean and temperate evergreens vs subtropical and
tropical evergreens), could be used for more data-rich traits. For
traits with fewer gradients (Table 1), plant functional types with
fewer observations were merged to form a broader functional
grouping.

2. Measurements of foliage photosynthetic capacity

For foliage photosynthetic characteristics, only studies explicitly
stating that the measurements were conducted at saturating or close to
saturating quantum flux density (Q), providing the photosynthetic
capacity, $A_{\text{cmax}}$, were incorporated in the database. In all
cases, measurement conditions, temperature, Q and ambient CO2
concentration ($C_{i}$) were included in the database. The estimates of
net assimilation rate conducted at ambient CO2 concentrations of
267–420 μmol mol$^{-1}$ (average ± SE = 354.2 ± 0.3 μmol mol$^{-1}$)
and temperatures of 15–35°C (average ± SE = 26.7 ± 0.1°C) with
the bulk of the measurements, 75%, conducted between tempera-
tures of 20 and 30°C) were defined as $A_{\text{cmax}}$, and used directly in
developing the correlations with light availability. This definition of
$A_{\text{cmax}}$ is inclusive and corresponds to that used commonly in global
databases of leaf structure and function (Niinemets, 1999; Wright
et al., 2004; Kattge et al., 2011). Although temperature signi-
ficantly alters $A_{\text{cmax}}$, the temperature response of $A_{\text{cmax}}$ has a broad
optimum over this temperature range (Cannell & Thornley, 1998;
Way & Yamori, 2014). We also argue that as long as the
measurement temperature is constant for different leaves measured
within the canopy light gradient, valid estimates of within-canopy
photosynthetic plasticity can be derived and these plastic estimates
are much less sensitive to variations in environmental drivers than is
$A_{\text{cmax}}$ itself (for the estimation of relative plasticity, see section IV.3).

Depending on whether the leaf dry mass per unit area ($M_{\text{A}}$) and/or
the leaf nitrogen content were available, mass-based photosynthetic
capacity ($A_{\text{M}}$; Eqn 1) and/or nitrogen use efficiency ($E_{\text{N}}$, Eqn 4)
were also calculated.

3. Standardized estimates of leaf biochemical potentials
   and photosynthetic capacity

Whenever the C3 photosynthesis model parameters of Farquhar
et al. (1980), $J_{\text{cmax}, A}$ and $V_{\text{cmax}, A}$, were reported, they were included
in the database. In this analysis, all model parameters are based on
intercellular CO2 concentration ($C_{i}$), as only one study included in
the analysis (Niinemets et al., 2006) provided estimates of
mesophyll diffusion conductance needed to calculate chloroplastic
CO2 concentration. As a result of the significant CO2 drawdown
between intercellular air space and chloroplasts (for a review, see
Flexas et al., 2012), the use of $C_{i}$ somewhat underestimates $V_{\text{cmax}}$
and $J_{\text{cmax}}$, as discussed in Notes S2.

The estimates of $J_{\text{cmax}, A}$ and/or $V_{\text{cmax}, A}$ measured at a leaf
temperature other than 25°C were converted to 25°C equivalent
estimates as explained in Notes S2. Mass-based estimates of
biochemical potentials at 25°C and apparent fractional nitrogen
allocations to Rubisco ($F_{\text{A}}$) and bioenergetics ($F_{\text{B}}$) were
further calculated according to Eqsns 5–7 for datasets reporting
$N_{\text{A}}$ or $M_{\text{A}}$ and $N_{M}$. As different Rubisco kinetic characteristics
have been used across the studies (Table S2) and this can potentially result in a
major bias (Rogers, 2014), we have converted all the $V_{\text{cmax}}$
estimates to a common set of Rubisco kinetic characteristics as
described in Notes S2. Given that the temperature relationships of
Rubisco kinetic characteristics from the study of Jordan & Ogren
(1984), as fitted by Harley et al. (1992) and Niinemets &
Tenhunen (1997), were used by the majority (60.5%) of the
studies, all other estimates were converted to this set of kinetic
characteristics. We also provide a procedure to convert $J_{\text{cmax}}$
estimates (Notes S2), but note that the $J_{\text{cmax}}$ values reported here were
not converted, as different Rubisco kinetics had only a minor impact on
$J_{\text{cmax}}$ estimation.

Overall, data on within-canopy variations in $V_{\text{cmax}}$ (or the slope
of the $A_{\text{cmax}}$ vs $C_{i}$ response curve) were reported in 42 studies, providing
information for 69 species, whereas $J_{\text{cmax}}$ estimates were reported in
44 studies, providing information for 45 species. Apart from the
CO2 response curves, inverse modeling techniques can be used to
estimate $V_{\text{cmax}}$ from $A_{\text{cmax}}$ (e.g. Niinemets & Tenhunen, 1997;
Niinemets, 1999; Niinemets et al., 1999c; Patrick et al., 2009).
However, for the inverse modeling, estimates of dark respiration
($R_{0}$) and $C_{i}$ are necessary, and, when available, these data, together
with estimates of stomatal conductance to water vapor ($g_{s}$), were
also included in the database. In addition, $J_{\text{max}}$ can be estimated from the CO$_2$-saturated net assimilation rate reported in some studies or from the effective quantum yield of photosystem II measured by chlorophyll fluorescence at saturating light (Evans, 1993; Schreiber et al., 1994; Niinemets et al., 1999a). Thus, additional estimates of $V_{\text{cmax}}$ and/or $J_{\text{max}}$ were derived from foliage photosynthetic characteristics as described in Notes S2, altogether resulting in estimates of foliage photosynthetic potentials ($V_{\text{cmax}}$ or $J_{\text{max}}$ or both) for 85 species from 92 studies.

When foliage biochemical potentials, $J_{\text{max}}$ and $V_{\text{cmax}}$ were ultimately available, a standardized estimate of $A_h$, $A_{\text{A,V}}$, was determined for a leaf temperature of 25°C and a $C_i$ of 251 µmol mol$^{-1}$ using the C$_3$ photosynthesis model of Farquhar et al. (1980), as described in Notes S2. This value of $C_i$ used for standardization corresponded to the average $C_i$ estimated across the studies reporting it. Standardized estimates of mass-based photosynthetic capacity, $A_{\text{M,V}}$, and nitrogen use efficiency, $E_{\text{N,V}}$, were also estimated. We note that these standardized estimates are based on the C$_3$ photosynthesis model, and do not have an analogous meaning for controls in C$_4$ photosynthesis. However, none of the studies conducted with C$_4$ species reported estimates of $C_i$ or stomatal conductance, and thus non-standardized estimates of $A_h$ are always reported for C$_4$ species through the article.

Although some of the $A_{\text{A,V}}$ values were derived from the measured estimates of $A_h$ (Notes S2), many studies reporting estimates of $J_{\text{max}}$ and $V_{\text{cmax}}$ did not report actual photosynthesis data. Thus, a large proportion of $A_{\text{A,V}}$ estimates were complementary to the measured $A_h$ values. Of the total of 3513 unique individual photosynthesis measurements in the entire dataset combining measured and standardized data, 729 were from $A_{\text{A,V}}$ (48 unique within-canopy gradients from, altogether, 324 photosynthetic capacity gradients). Analogously, 528 $A_{\text{M,V}}$ values from a total of 1823 (35 light gradients from 175) and 681 $E_{\text{N,V}}$ measurements from a total of 2411 photosynthetic nitrogen use efficiency estimates (43 gradients from 201) were unique.

IV. Methodology for analysis of within-canopy leaf trait variation: concepts and standardizations

1. Standardized estimates of light availability

We use the daily average incident integrated photosynthetically active quantum flux density on a horizontal surface during leaf development as an estimate of leaf light conditions ($Q_{\text{int}}$). As a standardized estimate, $Q_{\text{int}}$ was averaged for 50 d after the start of foliage development, or for the actual number of days since the start of foliage development for leaves either younger than 50 d or with an average leaf life-span of less than 50 d. This definition is based on observations indicating that foliage trait vs integrated average light relationships become stable 30–60 d after leaf formation in both woody and herbaceous canopies (Niinemets et al., 2004a; Niinemets & Keenan, 2012). Although foliage formation is initially rapid and light conditions within the canopy can undergo fast changes in developing herbaceous canopies (Niinemets & Keenan, 2012), leaf growth stops after the onset of generative growth, and leaves are exposed to relatively stable light conditions for the rest of the growing season (for a discussion on the possible effects of leaf turnover and reacclimation, see section VII.1 ). From a practical perspective, the estimation of light availability for shorter time periods in broad meta-analyses is associated with inherent uncertainties, in particular as a result of the estimation of dates of leaf formation (Notes S3), as well as day-to-day variations in cloudiness conditions at specific sites that cannot be captured well because of the relatively crude resolution of global databases of incident solar radiation (Notes S3). Details of the conversion of the different light measurements to $Q_{\text{int}}$ are reported in Notes S3.

As the incident quantum flux density is typically estimated by horizontal sensors and sampling locations in within-canopy studies characteristically corresponded to a cluster of leaves with a certain variation in foliar angle among the leaves within the given leaf cluster, in this analysis, the definition of $Q_{\text{int}}$ refers to the quantum flux density on a horizontal surface. Indeed, very few within-canopy studies have reported the actual leaf angles or leaf angle distributions in specific canopy positions (for some exceptions, see Hollinger, 1989, 1996; Niinemets, 1998; Ishida et al., 2001; Niinemets & Fleck, 2002; Niinemets et al., 2002; Cescatti & Zorer, 2003; Fleck et al., 2003). The use of the quantum flux density on a horizontal surface could be considered as an inherent limitation of the $Q_{\text{int}}$ concept. However, as the solar angle varies during the day, the effect of leaf angle on daily integrated leaf light receipt is relatively moderate (although it depends on the gap distribution in the canopy), with the largest effects on the share of light interception between the leaf upper and lower surface and on the distribution of the timing of interception of solar radiation with different intensities (Gutschick & Wieg, 1988; Cescatti & Niinemets, 2004; Valladares & Niinemets, 2007). Given this reasoning, four studies in which the light sensor was positioned parallel to the leaf surface (Oberbauer & Strain, 1986; Oberbauer et al., 1987; Lynch & González, 1993; Ackerly & Bazzaz, 1995) and eight studies in which the sensor was attached to the leaf surface (Chazdon & Field, 1987; Ackerly, 1992; Tjoelker et al., 1995; Traw & Ackerly, 1995; Osada et al., 2001; Han et al., 2006; Han & Chiba, 2009; Posada et al., 2009) were also incorporated into this analysis.

Although many within-canopy studies in the past have used the relative light intensity as the predictor variable in light availability vs leaf trait relationships (e.g. Hirose & Werger, 1987a; Anten et al., 1998b; Niinemets et al., 1998), we argue that, at a given relative light availability, there are strong variations in absolute incident light within and between seasons, and between different geographic locations. Indeed, significant fractions of light gradients were positioned at the lower (maximum $Q_{\text{int}}$ < 5 mol m$^{-2} \text{d}^{-1}$) and upper (minimum $Q_{\text{int}}$ > 15 mol m$^{-2} \text{d}^{-1}$) ends of the whole light range (Fig. S1), implying that the relative light estimates calculated for such truncated light ranges would be incomparable with broader light ranges. Furthermore, relative light availability is particularly inappropriate in glasshouse and growth chamber studies, where there can be major reductions in light as a result of absorption by enclosure materials and differences in the distance between the light source(s) and the plants (Niinemets & Keenan,
2. Fitting of leaf trait vs $Q_{\text{int}}$ relationships

Typically, leaf trait vs $Q_{\text{int}}$ relationships are non-linear (for sample relationships for representative forb, grass and broad-leaved deciduous woody species, see Fig. 1). Either non-linear functions in the form $y = ax^b$ or logarithmic functions in the form $y = \log(x)$ have been used to fit the data (e.g. Hirose & Werger, 1987a; Hirose et al., 1989; Anten et al., 1995, 1998a; Poorter et al., 2009, 2010; Niinemets & Keenan, 2012). However, initial data fitting demonstrated that these two-parameter functions did not always provide the best fits to the data. Thus, we have used a three-parameter monomolecular equation (Ceulemans et al., 1980; Causton & Dale, 1990):

$$y = y_{\text{max}} (1 - e^{-dF})$$

Eqn 8

where $y_{\text{max}}$ is the asymptotic value of the trait, and $d$ and $f$ are the empirical parameters. The monomolecular equation is more flexible than the two-parameter equations and can realistically fit strongly curved to almost linear relationships. Equation 8 was fitted to the data iteratively by minimizing the sum of the squares between the measured and the predicted trait values. In the case of variables declining asymptotically with increasing $Q_{\text{int}}$, this equation or a slightly modified equation (Eqn S4.1 in Notes S4) was employed. Further details of data fitting and adjustment of the statistical thresholds for the significance of relationships depending on the availability of the data are provided in Notes S4.

After parameterization, Eqn 8 or S4.1 was used to estimate the trait values at fixed values of $Q_{\text{int}}$ of 1, 3, 6, 12, 20, 30 and 40 mol m$^{-2}$ d$^{-1}$ to allow for comparison of trait estimates among plant functional types at standardized $Q_{\text{int}}$. Values of $Q_{\text{int}}$ of 1–3 mol m$^{-2}$ d$^{-1}$ correspond to typical light environments in the understory, 6–12 mol m$^{-2}$ d$^{-1}$ to moderately high light levels observed in medium-sized gaps and in mid-canopy, and 20–40 mol m$^{-2}$ d$^{-1}$ to high light environments observed in large gaps and in the upper canopy. Extrapolation beyond the data in calculating the trait estimates was constrained and did not exceed $\pm 0.7–5$ mol m$^{-2}$ d$^{-1}$, depending on the position in the $Q_{\text{int}}$ gradient and on the light range available for the given light gradient (Notes S5).

3. Definition and estimation of trait plasticity

Absolute trait values differ for different functional traits, and any given functional trait strongly varies among plant functional types. Furthermore, as a result of non-linearity, the trait plasticity also varies at different parts of the $Q_{\text{int}}$ gradient. This is relevant, as the
ranges of $Q_{\text{int}}$ available for different gradients varied among and within studies (Fig. S1). As a result of the non-linearity of light responses of foliage traits (Fig. 2; Poorter et al., 2009; Niinemets & Keenan, 2012), it is essential that trait comparisons are conducted over the same light range. To directly compare the light-dependent plasticity of any given trait among plant functional types at a
specified range of \( Q_{\text{int}} \), the relative light-dependent plasticity (\( P_{L,i} \)) of a given trait over the given light range \( l \), \( P_{L,i} \), was calculated as:

\[
P_{L,i} = \frac{v_{i+x} - v_i}{\Delta Q_{\text{int}}(v_{i+x} + v_i)/2},
\]

Eqn 9

where \( v_i \) is the trait value corresponding to a seasonal average quantum flux density of \( i \) (\( Q_{\text{int},i} \)) and \( v_{i+x} \) is the trait value corresponding to \( Q_{\text{int},i+x} \). \( P_{L,i} \) is defined as a relative plasticity because it is normalized with respect to the average trait value across the given light range, \( (v_{i+x} + v_i)/2 \). Such a normalization makes it possible to consider differences in absolute trait values between traits and between plant functional types for any given trait. The fold change (\( f_{i} \)) of a given trait over the given light range \( l \), \( v_{i+x}/v_i \), and \( P_{L,i} \) are related as:

\[
f_{i} = \frac{\Delta Q_{\text{int}}P_{L,i} + 2}{2 - \Delta Q_{\text{int}}P_{L,i}},
\]

Eqn 10

However, we note that \( f_{i} \) is not defined when \( v_i \leq 0 \).

The relative light-dependent plasticity was calculated over the \( Q_{\text{int}} \) ranges 1–3, 3–6, 6–12, 12–20, 20–30, 30–40 and 3–40 mol m\(^{-2}\) d\(^{-1}\) using the trait values, \( v_i \), estimated from the parameterized trait vs \( Q_{\text{int}} \) responses (Eqn 8, S4.1) employing the constraints specified in Notes S5.

4. Analysis of the quantitative importance of variations in constituent traits on photosynthetic plasticity

Equations 1–7 specify the functional relationships among structural, chemical and allocation traits and foliage photosynthetic potentials. The sources of light-dependent variation in composite traits, such as nitrogen content per area and photosynthetic potentials per unit leaf area, can be partitioned among the variations in constituent variables using a response coefficient analysis (Poorter & Nagel, 2000). The response coefficients for a given composite trait determined along a light gradient provide the fractions of variation caused by light-dependent variations of all the constituent traits (for the mathematics of the response coefficient analysis, see Notes S6). For example, the light-dependent variation in \( A_A \) as the product of \( A_M \) and \( M_A \) (Eqn 1) can be quantitatively ascribed to variations in \( A_M \) (\( A_A \) response coefficient for \( A_M \), \( R_{A_A}^{A_M} \)) and \( M_A \) (\( A_A \) response coefficient for \( M_A \), \( R_{A_A}^{M_A} \)) such that \( R_{A_A}^{A_M} + R_{A_A}^{M_A} = 1 \). The response coefficient for a given variable can be positive, when an increase in this characteristic is associated with an increase in the response variable. The response coefficient can also be zero, when a change in the trait does not affect the response variable, and it can be negative, when the trait increase is associated with a decrease in the response variable. The response coefficient analysis cannot be applied when the response variable is essentially invariant. Such an invariability in the response trait can reflect the invariability of the constituent traits, but can also result from opposite light responses of the constituent traits. For all light gradients in the current study, we calculated the response coefficients for all dependent variables in Eqns 1–7 using the trait values estimated at the maximum, \( v_i(Q_{\text{int},\text{max}}) \), and minimum, \( v_i(Q_{\text{int},\text{min}}) \), \( Q_{\text{int}} \) values present in the given dataset (section IV.1). Notes S6 provide further details of the estimation of the response coefficients.

5. Statistical analyses

In the case of fitting of the \( Q_{\text{int}} \) responses (section IV.2), the explained variance, \( r^2 \), was used as the goodness of fit. Kolmogorov–Smirnov tests were used to check the normality of traits and trait plasticity at specific \( Q_{\text{int}} \) values and the normality of trait response coefficients. Depending on the degree of positive skewness, square root or logarithmic transformations were used. In the case of negatively skewed distributions, the distributions were first reflected, and then transformed by square root or logarithmic transformations, and reflected again to restore the ranking. Finally, the traits, their plasticity and response coefficients were compared among plant functional types by one-way ANOVA followed by Tukey’s test, which is a conservative approach for unbalanced data. Different from multiple-factor ANOVA models, lack of balance does not affect the calculation of the sum of squares in one-way ANOVA (Montgomery, 2012). In addition, correlation and regression analyses were used to test for statistical relationships among traits and functional trait-specific means. All statistical relationships were considered to be significant at \( P<0.05 \).

V. Global variation in leaf characteristics through the canopies

1. Worldwide database on within-canopy variation in leaf structural, chemical and photosynthetic traits

We have constructed, to our knowledge, the most comprehensive database of within-canopy variations in 12 key foliage structural, chemical and physiological traits playing an important role in photosynthesis (see Table S1 and Notes S7 for data sources; Eqns 1–7). Altogether, the database includes 831 within-canopy gradients for 304 species belonging to 63 families and covering most vascular plant functional types forming dense vegetation canopies. To put the size of the database into perspective, the number of individual trait observations, 9231 for nitrogen content per dry mass (\( N_A \), 7390 for leaf dry mass per unit area (\( M_A \), 5757 for nitrogen content per dry mass (\( N_A \) and 3513 for photosynthetic capacity per unit leaf area (\( A_A \), is, depending on the trait, 2.8–4.7-fold greater than that for the global GloPnet database (Wright et al., 2004), and of similar magnitude for some traits (c. 68% for \( N_A \) and 115% for \( A_A \)) to the TRY database at the time of its publication (Katve et al., 2011). Thus, we consider this extensive database as an invaluable resource for understanding worldwide patterns in leaf functional differentiation through canopy light gradients.

Standardized estimates of average incident integrated light availability during leaf growth (\( Q_{\text{int}} \)) were determined for all leaves (section IV.1, Notes S3). Analysis of the distribution of all light gradients together (Fig. S1) underscores the fact that studies on
light-driven plasticity need to pay due attention to the within-canopy light range. A significant proportion of studies conducted so far include low to moderate light ranges, which can importantly limit the detection of the full light-driven plasticity. Nevertheless, c. 80% of light gradients included a minimum \(Q_{\text{int}}\) value of at least 10 mol m\(^{-2}\) d\(^{-1}\), and c. 60% of studies included a maximum \(Q_{\text{int}}\) value of at least 20 mol m\(^{-2}\) d\(^{-1}\) (Fig. S1). The limited light range can be a genuine limitation associated with particular stand growth conditions (open vs closed stands), species dispersal characteristics (understory vs overstory) and, in some species, lack of foliage formation or die-off in low light in the lower canopy when the upper canopy leaves have access to high light (e.g. Niinemets & Kull, 1998; Niinemets et al., 1998; Pons & Anten, 2004; Boonman et al., 2006).

Poorter et al. (2009, 2010) coped with the limited light range by standardizing all measurements conducted at different light environments (understory–gap light gradient) to a common moderately high integrated light estimate of 8 mol m\(^{-2}\) d\(^{-1}\). However, all light gradients that do not include this light range, a significant fraction in our worldwide database (Fig. S1), would be left out when using this method. Thus, here, we have developed an alternative data distillation strategy by fitting the data over the available light range and deriving foliage light-dependent plasticity estimates (Eqn 9) for finite light ranges according to predefined rigorous criteria for available minimum and maximum \(Q_{\text{int}}\) and \(Q_{\text{int}}\) range (section IV.3 and Notes S5). This more inclusive strategy makes it possible to use most of the data, with only a minor fraction of the data rejected as a result of light ranges that are too narrow (between less than 1% to a few percent for different traits).

2. Overall variations in leaf traits among plant functional types

Across the whole database, all the studied traits were characterized with extremely high variability of c. 20- to more than 100-fold for different traits (Table 1). Combinations of traits strongly varied across plant functional types with woody plant functional types being characterized by high \(M_A\) and low \(N_M\), \(A_M\) and \(E_N\) (for an illustration of average trait values standardized to a moderately high \(Q_{\text{int}}\) of 12 mol m\(^{-2}\) d\(^{-1}\) in different plant functional types, see Fig. 2) and with analogous patterns for \(V_{\text{max,}M}\), \(J_{\text{max,}M}\), \(F_b\) and \(F_k\) (data not shown). For plant functional type averaged and light standardized (\(Q_{\text{int}} = 12\) mol m\(^{-2}\) d\(^{-1}\)), there were strong negative correlations of \(A_M\) vs \(M_A\) (Fig. 2b), \(N_M\) vs \(M_A\) (Fig. 2f) and \(E_N\) vs \(M_A\) (\(\rho^2 = 0.894, P < 0.001\)) and positive correlations among \(A_M\) vs \(N_M\) (Fig. 2c) and \(A_M\) vs \(E_N\) (\(\rho^2 = 0.904, P < 0.001\)). These scaling relationships reflect the leaf economics spectrum, i.e. co-occurrence of combinations of high \(M_A\), low \(N_M\) and \(A_M\) in the low return end of the spectrum (woody) vs low \(M_A\), high \(N_M\) and \(A_M\) in the high return end of the spectrum (Wright et al., 2004). As a result of these compensatory relationships, \(N_A\) (Fig. 2a) and \(A_A\) (Fig. 2d) were much less variable among the plant functional types than were \(M_A\) (Fig. 2b), \(N_M\) (Fig. 2c), \(A_M\) (Fig. 2e) and \(E_N\) (Fig. 2f) (Table S3). For the broad herbaceous vs woody comparison, the average values of \(N_{\text{int}}\), \(A_{\text{int}}\), \(N_A\) and \(A_A\) were significantly greater for herbaceous species, whereas the average values for \(M_A\) and \(N_A\) were greater for woody species (\(P < 0.001\) for all comparisons, except for the comparison of \(A_A\), where \(P > 0.01\)).

Comparisons among individual plant functional types further corroborated these broad patterns. The averages for \(M_A\), \(N_M\), \(A_M\) and \(E_N\) differed significantly among all plant functional types, but \(N_A\) differed among fewer herbaceous and woody plant functional types, and \(A_A\) was significantly lower only in needle-leaved evergreens than in most other plant functional types (Table S3). This low variability in \(N_A\) and invariability of \(A_A\) among plant functional types is consistent with the trait variation in the worldwide leaf economics spectrum (Wright et al., 2004), and indicates that a given value of \(A_A\) and \(N_A\) can result from different combinations of underlying traits.

VI. Trait scaling with light, plasticity and quantitative limitations

1. Non-linear scaling of foliage traits with light and the strength of light relationships

All of the traits analyzed here are characterized by strong light-dependent within-canopy variations, with particularly large light-dependent increases observed for the area-based traits \(A_A\), \(J_{\text{max,}A}\), \(V_{\text{max,}A}\), \(N_A\) and \(M_A\), followed by mass-based photosynthesis potentials and nitrogen allocation traits (Table 1). The extensive analysis carried out here demonstrates that the \(Q_{\text{int}}\) vs structural, chemical and photosynthetic trait relationships are characteristically non-linear (for sample relationships, see Fig. 1), implying that, for incomplete light gradients, data fitting and analysis over finite light ranges is essential (Notes S4). Here, the data were fitted by non-linear three-parameter equations (Eqn 8 or S4.1) that improved the degree of explained variance compared with conventional logarithmic or power equations (for sample fits of key leaf traits, see Fig. 1). Low \(r^2\) values of regression fits typically truly reflected the trait invariability through the canopy (Fig. 1b,c) rather than extensive unexplained within-canopy fluctuations of the given trait.

Analysis of the variations among the traits according to the degree of explained variance (\(r^2\)) in leaf trait vs \(Q_{\text{int}}\) relationships demonstrates that the area-based characteristics, \(N_A\) (Fig. 3a) and \(A_A\) (Fig. 3d), are universally strongly associated with \(Q_{\text{int}}\) in all plant functional types. These two traits are followed by \(M_A\), which, however, is weakly dependent on \(Q_{\text{int}}\) in grasses (Fig. 3b). The relationships are strong across plant functional types, although herbaceous species have, on average, higher \(r^2\) for both \(N_A\) (\(P < 0.02\)) and \(A_A\) (\(P < 0.002\)), and woody species for \(M_A\) (\(P < 0.001\)), vs \(Q_{\text{int}}\) relationships. In the case of \(A_A\) and \(N_A\), this reflects lower \(r^2\) values in needle-leaved evergreens (Fig. 3a,d; Table S3), whereas, in the case of \(M_A\), the significant herbaceous vs woody contrast reflects primarily the greater \(r^2\) values in winter-deciduous species relative to annual forbs and grasses (Fig. 3b; Table S3).

Different from these three traits, there are major differences in \(r^2\) for \(N_M\) (Fig. 3c), \(A_M\) (Fig. 3c) and \(E_N\) (Fig. 3f) among herbaceous
and woody species ($P < 0.001$ for all three traits; for statistical differences among individual herbaceous vs woody plant functional types see Table S3), with woody species having much weaker $Q_{\text{int}}$ dependences for these traits (Fig. 3c,e,f). In addition, perennial forbs also had a lower average $r^2$ than annual forbs for $N_M$ vs $Q_{\text{int}}$ relationships (Fig. 3c; Table S3).

Another way to gain an insight into the strength of leaf trait vs $Q_{\text{int}}$ relationships is to look at the percentage of statistically non-significant relationships and at significant, but negative relationships. Of course, in the case of statistically non-significant relationships developed over limited canopy light range, we cannot ultimately say whether there could have been a significant response over a more extensive light range. Nevertheless, comparisons of the light response of different traits analyzed in the same study over the same light range, and across studies (e.g. Fig. 1), can still provide appropriate information on the overall differences in the strength of the light relationships. In the case of $N_A$ and $A_A$, there were no statistically significant negative relationships, and the number of non-significant $Q_{\text{int}}$ responses was, in most cases, low, a few percent, with the exception of somewhat larger percentages of $c. 10--15\%$ in perennial $C_3$ grasses and evergreen broad-leaved tropical and needle-leaved species for $N_A$ (Fig. 3a) and in evergreen broad-leaved tropical species for $A_A$ (Fig. 3d). In the case of $M_A$ (Fig. 3b), grasses had a large percentage of non-significant and negative relationships, almost $50\%$, whereas, in annual forbs, $c. 13\%$ of relationships were negative.

Fig. 3 (a–f) Variation in the degree of explained variance ($r^2$) in leaf trait vs integrated light ($Q_{\text{int}}$) relationships (Eqns 8 and S4.1 in Notes S4) among different foliage structural, chemical, allocational and photosynthetic traits and among different plant functional types illustrated by the box plots (see Fig. 2 for a detailed explanation). The first number next to each box provides the percentage of statistically non-significant relationships (see Notes S4 for the significance limits), whereas the second number provides the percentage of statistically significant negative relationships. Classification of data according to plant functional types as in Fig. 2. For significantly different $r^2$ values among plant functional types, see Table S3.
Different from these three traits, lack of statistical significance was much more frequent for $N_M$ (Fig. 3c), $A_M$ (Fig. 3e) and $E_N$ (Fig. 3f) vs $Q_{int}$ relationships, in particular for woody plant functional types. Among woody plant functional types, the percentages of negative or non-significant relationships were c. 49–70% for $N_M$, c. 35–50% for $A_M$ and c. 50% for $E_N$. Although the $Q_{int}$ responses for these three traits were much stronger for herbaceous plant functional types, 33% of $N_M$ relationships for perennial forbs and 16% for those in grasses were not significant (Fig. 3c). We note that because of the limited number of observations available for different plant functional types, not all comparisons among functional types can be made for all traits. Nevertheless, these data demonstrate important qualitative differences in leaf trait vs light relationships for different plant functional types, in particular among herbaceous vs woody species.

2. Negative trait plasticity: why does it occur?

According to the functional relationships linking leaf structural, chemical and allocational traits to leaf photosynthetic capacity (Eqs 1–7), maximization of foliage photosynthetic capacity within the canopy light gradients suggests that all of these leaf traits should increase with increasing $Q_{int}$. Such broad trends for all traits are apparent across all the data (Table 1), but, nevertheless, a certain number of negative $Q_{int}$ relationships were observed for $M_A$, $A_M$, $N_M$ and $E_N$ (Figs 1, 3). Although most of these statistically significant negative trait vs $Q_{int}$ relationships typically exhibited a minor negative trend, implying that the overall effects of the negative variations in these traits on the target variable $A_M$ were moderate, the question remains as to what are the sources of such puzzling negative scaling trends.

In the case of $M_A$, we believe that the negative $Q_{int}$ responses can be explained by three different mechanisms dependent on the location of growing leaf tissues in the canopy, the timing of foliage growth and the timing and rate of growth of neighboring individuals in the canopy. First, in the temperate grass Carex aquatilis, which is characterized by a particularly strong negative response of $M_A$ to $Q_{int}$ (Hirose et al., 1989; Pons et al., 1993), foliage extends from the base of the leaf. Thus, the basal parts of long extended leaves have to support the entire leaf. Therefore, the leaves increase in thickness and have a more pronounced midrib towards the base of the plant, resulting in a greater $M_A$ at a lower $Q_{int}$ in the bottom of the canopy. Second, as explained in Notes S4, in fast-growing canopies, characterized in herbs, but also occasionally in woody species (Vapaavuori & Vuorinen, 1989; Vapaavuori et al., 1989; Dickmann et al., 1990; Medhurst & Beadle, 2005), continuously forming leaves at the top of the canopy, there can be important interactions among leaf age and $Q_{int}$. Although all leaves may have been formed at high light, leaves in the extending upper canopy can be developmentally younger and exhibit a lower $M_A$ at the time of sampling than fully mature leaves in the lower canopy. For canopies in which the formation of new leaves extends into the late season, it is also plausible that above-canopy $Q_{int}$ is higher for leaves formed in the lower canopy than for leaves forming later. Finally, in mixed fast-growing canopies with a developing size hierarchy of individuals (Anten & Hirose, 1998; Anten et al., 1998a; Hikosaka et al., 1999) or with differences in growth rate of constituent species (Hirose & Werger, 1994, 1995), the leaves formed early have been exposed to the same $Q_{int}$. However, later during the course of the stand development, plant individuals growing more rapidly will shade the developing leaves in individuals growing more slowly. This implies that, in these less competitive individuals, early- and late-formed leaves have been exposed to different $Q_{int}$ values.

We argue that negative $Q_{int}$ relationships for other leaf traits can also be explained, in many cases, by developmental effects. However, these mechanisms cannot explain negative $Q_{int}$ relationships for flush-type species either forming all leaves simultaneously at the beginning of the growing season (the bulk of winter-deciduous species) or having the leaf flush periods broken by sustained periods of new leaf formation (evergreen needle-leaved species, many tropical canopy species). In these species, it has been argued that a moderate reduction in $N_M$ accompanied by reductions in $A_M$ can reflect dilution of foliar nitrogen as a result of enhanced costs of structural carbohydrates and lignin (e.g. Niinemets & Kull, 1998) to cope with potentially more severe water stress in the upper canopy (Niinemets et al., 1999b, 2004b; Hubbard et al., 2002; Aasamaa et al., 2004; Sellin & Kupper, 2004). Although water stress can be especially significant in plant stands growing in inherently drier habitats, limited hydraulic conductivity of stems and branches can constrain water transport to the upper canopy in clear days, even when soil water availability is not limiting (Joyce & Steiner, 1995; Brodribb et al., 2005; Renninger et al., 2006; Ewers et al., 2007; Peltoniemi et al., 2012). Enhanced water stress is further expected to reduce intercellular CO$_2$ concentration more strongly in the upper canopy (Aasamaa et al., 2004; Niinemets et al., 2004b), reducing the estimates of $A_M$ and $E_N$. Furthermore, limited water transport also alters the propagation of root-borne hormonal signals by cytokinins and abscisic acid, which can importantly modify the...
development of leaf photosynthetic capacity (Pons et al., 2001; Boonman et al., 2009). Taken together, we suggest that a complex array of factors can be responsible for negative scaling of foliage traits with increasing $Q_{int}$ in the canopy. Some of these factors, for example, differences in leaf age, could sometimes be controlled by skilled sampling designs. However, this is not necessarily possible in canopies with fast leaf turnover, where there are inherent interactions between light environment, leaf age and leaf turnover.

3. Implications of trait non-linearity for trait plasticity

As a result of non-linearity, the relative trait plasticity calculated over a finite light range ($P_L$, Eqn 9) decreases with increasing $Q_{int}$ range (Figs 4, S2). The pertinent question is what is the most appropriate light range for comparison of the plasticities of different traits and plasticities of any given trait among plant functional types. Although the lowest light range, 1–3 mol m$^{-2}$ d$^{-1}$, used for $P_L$ calculation is apparently the most plastic (Figs 4, S2), only slight inaccuracies in $Q_{int}$ estimation have a large effect on the plasticity estimate and therefore, can make this light range overly sensitive to such errors. However, the traits become light saturated at the higher light ranges of 20–30 and 30–40 mol m$^{-2}$ d$^{-1}$ (Figs 4, S2), implying that the variability of plasticity over this light range is too low for any comparison among plant functional types. Furthermore, as a result of limitations in light range coverage (Fig. S1), the use of both the lower and higher light ranges, and the overall light range of 3–40 mol m$^{-2}$ d$^{-1}$, would lead to the neglect of a significant part of the light gradients. Thus, for the following analysis, we have used the $P_L$ estimates for the moderately high light range of 6–12 mol m$^{-2}$ d$^{-1}$. Over this

Fig. 5 (a–f) Comparison of relative trait plasticity ($P_L$, Eqn 9) estimated for a moderately high integrated light ($Q_{int}$) range of 6–12 mol m$^{-2}$ d$^{-1}$ within and among key plant functional types. As defined, $P_L$ values are directly comparable for different traits and for the same traits for light gradients with varying average trait values. The box plots for each trait and each plant functional type characterize the distribution of the given trait. Plant functional types and details of box plots are given in Fig. 2. The total number of gradients for each trait is reported in Table 1, and statistically significant plasticity differences among plant functional types are demonstrated in Table S3.
light range, just before flattening the trait vs $Q_{\text{int}}$ responses, foliage traits still respond strongly to $Q_{\text{int}}$, and this light range was also present in the bulk of the light gradients. Indeed, analysis of differences in trait plasticity for $A_L$, $N_A$ and $M_A$ among woody and herbaceous species indicated that the differences were in the same direction for these three traits for all light ranges, with the exception of only the highest light range of 30–40 mol m$^{-2}$ d$^{-1}$ for $A_L$ (Fig. 4). Thus, we conclude that the use of the selected light range 6–12 mol m$^{-2}$ d$^{-1}$ is representative to gain an insight into trait and functional type differences in trait plasticities.

4. Does plant functional type determine the light-dependent plasticity of different traits?

The analysis of light-dependent plasticity over the moderately high light range of 6–12 mol m$^{-2}$ d$^{-1}$ highlights important variations in plasticity among different traits and for given traits across plant functional types (Fig. 5). First, this analysis underscores the overall greater light-dependent plasticity of the area-based traits $A_L$ (Fig. 5d), $N_A$ (Fig. 5a) and $M_A$ (Fig. 5b) compared with the mass-based traits $M_M$ (Fig. 5c), $A_M$ (Fig. 5e) and the allocation trait $E_N$ (Fig. 5f), confirming the evidence of overall differences in the variability of traits over the whole light range (Table 1). However, there are major differences among plant functional types in the plasticity of individual traits. For the broad herbaceous vs woody comparison, there is no clearcut difference in $N_A$ plasticity ($P > 0.9$), but a finer scale analysis demonstrates several significant differences in $N_A$ plasticity among plant functional types. In particular, $N_A$ plasticity in annual C$_3$ grasses tends to be greater, and that in needle-leaved evergreens smaller, than in most other plant functional types (Fig. 5a, Table S3).

The broad herbaceous vs woody comparison suggests that herbaceous species are more plastic in all other traits, except for $M_A$, which is more plastic in woody species ($P < 0.001$ for all comparisons). However, at a finer scale, $M_A$ (Fig. 5b) and $N_M$ (Fig. 5c) plasticities in perennial forbs are similar to those in woody species, and $A_N$ and $E_N$ plasticities for forbs (perennials and annuals pooled because of a limited sample size) are similar to those in woody plant functional types with a few exceptions (Table S3). In the case of $A_L$ plasticity, key differences are a greater plasticity in perennial C$_3$ grasses than in all other woody plant functional types, and a greater plasticity in annual forbs than in evergreen tropical and needle-leaved species (Fig. 5d, Table S3). Surprisingly, there are very few significant differences in these key traits among woody plant functional types. Only $N_A$ plasticity of evergreen needle-leaved species is significantly less than that for winter-deciduous and temperate evergreen broad-leaved species, and $M_A$ plasticity in winter-deciduous broad-leaved species is greater than that in evergreen needle-leaved and tropical broad-leaved species (Fig. 5a; Table S3). Clearly, the small number of observations for the plasticity of some traits, such as $M_A$ in grasses (Fig. 5b), limits the generality of our conclusions, but, nevertheless, the broad differences among herbaceous vs woody species and among more frequently sampled functional types stand out and emphasize that light-dependent plasticity truly varies among plant functional types.

Fig. 6 Quantitative partitioning of the light-dependent variations in nitrogen content per area ($N_A$) as a result of leaf dry mass per unit area ($M_A$) and nitrogen content per dry mass ($N_M$, Eqn 3) within and among plant functional types according to the response coefficient analysis (a), and the correlations between the relative light-dependent plasticities in $N_A$ and $M_A$ for the light range of 6–12 mol m$^{-2}$ d$^{-1}$ ($P_L$, Eqn 9) in different plant functional types (b). The response coefficient ($R_c$) for a given trait (section IV.4 and Notes S6, Eqns S6.1–S6.4) provides the fraction of light-dependent variance in the target variable as a result of the light-dependent variation in the given trait. The response coefficients of $N_A$ for $M_A$ and $N_M$ sum up to 1. Positive $R_c$ values indicate a positive effect of the given trait on the target variable, whereas negative $R_c$ values indicate a negative effect. $R_c$ values close to zero indicate that light-dependent variation in the given variable has a minor effect on the variation of the target variable. Figure 2 provides the specifics of the box plots as used in (a) and the logic of the separation of plant functional types in (a) and (b). Each symbol in (b) corresponds to a different light gradient. For better visual assessment, the outliers in the box plots were suppressed in (a), and the data for the perennial grass Carex acutiformis exhibiting strongly positive $P_l$ values between −0.027 and −0.063 for $M_A$ (see section VI.2 and Notes S4) were suppressed in (b). (a) Statistical significance among the response coefficients for the given plant functional type (paired t-tests): *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significantly different. (b) The functional type-specific regressions were compared by SMATR version 2.0 which provides tests for differences among slopes, intercepts and elevations of standardized major axis regressions (Warton et al., 2006). A common slope test indicated that the $N_A$ vs $M_A$ plasticity relationship in herbs had a greater elevation ($P < 0.001$ for all data and $P = 0.02$ for a dataset in which C. acutiformis data exhibiting large negative values of $M_A$ plasticity were removed).
5. Quantitative partitioning of within-canopy photosynthetic variations among the constituent traits

The quantitative limitation analysis (section IV.4 and Notes S6, Eqns S6.1–S6.4) makes it possible to separate the sources of variation of any given target trait among its components, thereby providing informative insight into the functional trait relations (Eqns 1–7). The response coefficients describing variation in $N_A$ (Eqn 3), the $N_A$ response coefficient for $M_A$, $R_{MN}$, and that for $N_M$, $R_{NM}$, strongly varied among plant functional types (Fig. 6a). In the case of annual forbs and grasses, $R_{NM}$ was significantly larger than $R_{MN}$, indicating that the light-dependent variation in $N_A$ was primarily dependent on variation in $R_{NM}$. In perennial forbs, $R_{NM}$ and $R_{MN}$ did not differ statistically, suggesting that the control on $N_A$ was shared by both constituent variables $N_M$ and $M_A$. Different from the herbaceous species, $R_{NM}$ was much larger than $R_{MN}$ in all woody plant functional types, indicating that the variation in $N_A$ was mainly driven by changes in $M_A$. Alternative analysis of co-variation of light-dependent trait plasticity indicates that the relationship between $M_A$ and $N_A$ plasticities falls almost to a 1 : 1 line in woody species, whereas herbaceous species have a stronger increase in $N_A$ plasticity with increasing $M_A$ plasticity, demonstrating the additional role of $N_M$ plasticity in shaping $N_A$ plasticity in herbaceous species (Fig. 6b).

By focusing on the quantitative light-dependent controls on $A_A$ by $A_M$ and $M_A$ (Eqn 1), we again indicate that the $A_A$ control is mainly attributable to changes in $A_M$ in herbaceous plant functional types, whereas changes in $A_A$ are dominated by $M_A$ in all woody plant functional types (Fig. 7a). In a similar manner, when the variation in $A_A$ is deconvoluted among the variations caused by $N_A$ and $E_N$ (Eqn 4), the contribution of the variations in $E_N$ is greater in herbaceous plant functional types and the contribution of the variations in $N_A$ is greater in woody plant functional types (Fig. 7b). Ultimately, when the changes in $A_A$ caused by alterations in $E_N$ and changes in the components of $M_A$ and $N_M$ (Fig. 7a, Eqn 4) are analyzed together, it becomes evident that, in forbs, $A_A$ changes primarily as a result of changes in both $M_A$ and $E_N$, whereas, in grasses, as a result of changes in both $E_N$ and $N_M$ (Fig. 7c). In woody species, $A_A$ variation is most strongly determined by changes in $M_A$, whereas the contributions of $N_M$ and $E_N$ are equally low in all cases, except for evergreen tropical woody species, where changes in $E_N$ contribute somewhat more to $A_A$ change than do changes in $N_M$ (Fig. 7c).

Analyses of the sources of variation in biochemical photosynthesis potentials (Eqns 5–7) provide qualitatively similar evidence to the analyses of $A_A$ controls, although, because of the limited number of observations for several plant functional types, the results are less clearcut (Fig. 8). Especially in herbaceous species, when the effects of variations in all three contributors are analyzed together, variations in $M_A$ contribute somewhat more strongly to changes in the area-based photosynthetic potentials than do changes in the fractional nitrogen investment (Fig. 8c). This latter result apparently contradicts the greater role of variations in mass-based photosynthesis potentials relative to $M_A$ changes in
herbaceous species (Fig. 8a). As the light-dependent plasticity for different traits varies among different herbaceous plant functional types (Fig. 5), gaining an insight into this discrepancy would require the examination of functional controls among different herbaceous plant functional types as more data become available in the future.

6. Why is there invariability or variability of different traits within and among canopies?

The extent to which light-dependent changes in foliage photosynthetic potentials depend on variations in different underlying traits, and how these controls vary among plant functional types, have been poorly characterized to date. Analysis of the global within-canopy trait variations highlights that $A_\text{N}$ and foliage biochemical photosynthesis potentials increase with increasing $Q_{\text{int}}$ similarly in canopies of all major plant functional types (Table 1; Fig. 5d). However, detailed analyses of all constituent traits based on differences in $r^2$ values, the number of non-significant or negative relationships (Fig. 3), differences in trait plasticities (Figs 4, 5, S2) and quantitative limitation analyses (Figs 6–8) collectively demonstrate that a similar $A_\text{N}$ plasticity is associated with different combinations of plasticities of underlying traits in different plant functional types. In particular, in woody plant functional types, $A_\text{N}$ increases with increasing light availability mostly because of increasing $M_\text{A}$, whereas, in herbaceous plant functional types, the increase in $A_\text{N}$ is shared between changes in $N_\text{M}$ and $E_\text{N}$ (Figs 7, 8).

We argue that these differences reflect fundamental contrasts in the functioning of different types of plant canopies. In the case of herbaceous species with high leaf turnover, the whole canopy is formed during a single growing season and the canopy grows continuously until the formation of inﬂorescences. Thus, all leaves have been exposed to high light availability at least at some part during their development. In addition to canopy vertical expansion from the top, senescence is induced in older shaded leaves in the lower canopy. There is ample experimental evidence that shading in fast-growing species with fast leaf turnover, especially shading of individual leaves, can introduce programmed cell death, leading to rapid declines in leaf nitrogen content, leaf photosynthetic capacity and, ultimately, to leaf abscission (Burkey & Wells, 1991; Pons & Pearcy, 1994; Ackerly & Bazzaz, 1995; Ono et al., 2001; Vos & van der Putten, 2001; Boonman et al., 2006). Although $M_\text{A}$ increases with increasing $Q_{\text{int}}$ in the canopies of several herbaceous species (Figs 1a, 5b), the increase is much less than the corresponding change in $N_\text{M}$ through the canopy (Figs 1b, 5c), such that the increase in $N_\text{A}$ (Figs 1c, 5a) is mainly dependent on the within-canopy gradient in $N_\text{A}$. Furthermore, $A_\text{M}$ (Figs 1e, 5e) and $E_\text{N}$ (Figs 1d, 5f) strongly vary through the canopies of herbaceous species. All of these changes are compatible with shade-induced reallocation of nitrogen from components of photosynthetic machinery to leaves in upper high-light-exposed leaves, as well as with partial reacclimation to changed light conditions in shaded leaves. Thus, the overall evidence suggests that the within-canopy $A_\text{N}$ acclimation in herbaceous canopies is a highly dynamic active process.

The situation is different in flush-type woody species, such as most winter-deciduous species and evergreen needle-leaved species, temperate and Mediterranean evergreens and many tropical evergreens, where periods of intensive leaf formation are broken...
by dormancy or rest periods. In these species, all leaves or the given leaf flush are formed essentially at the same time, and the leaves are exposed to different light environments during their entire life-span. All leaves are maintained through the entire season and there is little change in leaf nitrogen content and photosynthetic capacity until the onset of leaf senescence, which occurs at the end of the growing season in deciduous species (Niinemets et al., 2004a; Grassi et al., 2005) or typically somewhat before new leaf flush in evergreen species (Grubb, 1996; Lusk et al., 2003; Portillo-Estrada et al., 2013). In these species, there is a strong variation in $M_A$ through the canopy (Figs 1a, 5b), whereas $N_M$ (Figs 1b, 5c), $A_M$ (Figs 1e, 5e) and $E_N$ (Figs 1d, 5f) vary much less, and, accordingly, the within-canopy changes in $A_A$ are primarily determined by changes in $M_A$. This implies that the formation of the within-canopy gradient in $A_A$ in these species is largely passive, reflecting the acclimation of $M_A$ to the canopy light gradient during leaf development. Nevertheless, these species possess a certain capacity to acclimate to sudden modifications in the light environment that can occur on gap formation in the canopy, but these changes are typically slow and constrained by the inability for structural modifications once the leaves have developed fully (e.g. Naidu & DeLucia, 1998; Niinemets et al., 2003; Oguchi et al., 2005).

Of course, there are mixed response patterns between these two extreme mechanisms of acclimation to within-canopy light gradients (Fig. 5). In particular, within-canopy acclimation in fast-growing deciduous and evergreen woody canopies with continuous canopy growth approximates the acclimation in herbaceous canopies. In such stands, foliage developed earlier becomes increasingly shaded by the expanding canopy, leading to strong leaf age and light gradients (Vapaavuo & Vuorinen, 1989; Vapaavuori et al., 1989; Noormets et al., 1996; Kull et al., 1998; Weih, 2009). Similar to herbaceous species, $M_A$ is relatively invariable in fast-growing woody canopies, and the within-canopy changes in $N_A$ are chiefly determined by a strong gradient in $N_M$ (Vapaavuo & Vuorinen, 1989; Vapaavuori et al., 1989; Noormets et al., 1996; Kull et al., 1998), whereas the within-canopy variations in $A_A$ are driven by $Q_{dis}$-dependent increases in $A_M$, $N_A$ and $E_N$ (Vapaavuo & Vuorinen, 1989; Vapaavuori et al., 1989).

**VII. Conclusions: the economics spectrum for the within-canopy plasticity**

Across all data incorporated in the database, a vast variability in key leaf functional traits was observed (Table 1, Fig. 2). This vast variability is consistent with the worldwide leaf economics spectrum linking plant species with fast leaf turnover and high $N_M$, $A_M$ and low $M_A$ at the high end of the spectrum, and plant species with low leaf turnover and low $N_M$, $A_M$ and high $M_A$ at the low return end of the spectrum (Wright et al., 2004, 2005; Westoby et al., 2013). The concept of the leaf economics spectrum has been criticized on the grounds that within-canopy variation is primarily driven by changes in area-based leaf traits (Lloyd et al., 2013). However, the worldwide analysis here suggests that the concerns expressed with regard to the appropriateness of mass-based traits in the context of light acclimation of photosynthetic potentials are not valid. Indeed, there is a spectrum of trait responses to the within-canopy light gradient within the leaf economics spectrum. Although, in species at the low return end of the economics spectrum, the acclimation response of $A_A$ is driven by changes in $M_A$, in the canopies of herbaceous species at the high return end, $A_A$ varies mainly as a result of changes in $N_M$ and in fractions of nitrogen invested in photosynthetic machinery. Indeed, compared with the worldwide variation in leaf traits, the variation in within-canopy acclimation is much compressed, reflecting the opposite scaling of leaf functional traits (e.g. the insets in Fig. 2b, f) and different combinations of traits varying through plant canopies in different plant functional types. These compensatory responses lead to bafflingly low variability in $A_A$ plasticity across plant functional types (Fig. 5d). However, this ‘invariability’ of $A_A$ plasticity is a result of fundamentally different structural, chemical and allocational controls in plant functional types with varying leaf turnover.

According to the ‘full optimality’ hypothesis of within-canopy photosynthetic acclimation, $A_A$ should be directly proportional to $Q_{dis}$. However, such a full optimality has never been found. Lack of ‘full optimality’ has been suggested to reflect constraints on photosynthetic acclimation (Niinemets & Valladares, 2004; Dewar et al., 2012; Niinemets, 2012; Peltoniemi et al., 2012). We suggest that plant canopies fundamentally differ in the suite of operating constraints. A limited rate of nitrogen reallocation and a limited share of nitrogen investments among leaf proteins of the photosynthetic apparatus and the rest constitute such constraints in species with high leaf turnover, whereas the structural construction limits define the constraints for within-canopy photosynthetic acclimation in species with low leaf turnover. We argue that studies attempting to resolve the controls on within-canopy variations in $A_A$ should analyze variations in structural, chemical and allocational traits collectively.

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## Supporting Information

Additional supporting information may be found in the online version of this article. Additional references can be found here.

**Fig. S1** Cumulative proportion of within-canopy light gradients with the maximum average seasonal integrated quantum flux density (*Q*<sub>int,max</sub>) equal to or greater than the given threshold value *Q*<sub>int,max</sub> and the cumulative proportion of light gradients with the minimum *Q*<sub>int</sub> equal to or smaller than the given threshold value *Q*<sub>int,min</sub>.

**Fig. S2** Sample relationships of the variation of relative light-dependent plasticity with the integrated light range in forbs and winter-deciduous trees.

### Table S1

| List of species, species classification, trait coverage in the database, light gradient characteristics and data sources of the global within-canopy trait database |

### Table S2

| Kinetic characteristics of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) used across the studies and standardization factors used for the maximum Rubisco carboxylase activity |

### Table S3

| Results of statistical comparisons among plant functional types |

### Notes S1

| Leaf area conversions for needle-leaved species. |

### Notes S2

| Estimation of biochemical photosynthesis model parameters, their standardization, consideration of gross photosynthesis data and estimation of standardized photosynthetic capacity. |

### Notes S3

| Additional methods for the estimation of the integrated quantum flux density (*Q*<sub>int</sub>). |

### Notes S4

| Constraints employed in fitting non-linear relationships, consideration of non-asymptotic responses and statistical thresholds. |

### Notes S5

| Constraints applied in the calculation of relative plasticity and trait values at given light levels, determination of normalized plasticity, and consideration of plasticity estimates for measured and standardized photosynthetic traits. |

### Notes S6

| Quantitative limitation analysis: definition of response coefficients and computation details. |

### Notes S7

| Additional references cited in the online supporting information. These references form an integral part of the article. |

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