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Leaf age dependent changes in within-canopy variation in leaf functional traits: a meta-analysis

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Abstract

Within-canopy variation in leaf structural and photosynthetic characteristics is a major means by which whole canopy photosynthesis is maximized at given total canopy nitrogen. As key acclimatory modifications, leaf nitrogen content (N_A) and photosynthetic capacity (A_A) per unit area increase with increasing light availability in the canopy and these increases are associated with increases in leaf dry mass per unit area (M_{Δ}) and/or nitrogen content per dry mass and/or allocation. However, leaf functional characteristics change with increasing leaf age during leaf development and aging, but the importance of these alterations for within-canopy trait gradients is unknown. I conducted a meta-analysis based on 71 canopies that were sampled at different time periods or, in evergreens, included measurements for different-aged leaves to understand how within-canopy variations in leaf traits (trait plasticity) depend on leaf age. The analysis demonstrated that in evergreen woody species, M_A and N_A plasticity decreased with increasing leaf age, but the change in A_A plasticity was less suggesting a certain re-acclimation of A_A to altered light. In deciduous woody species, M_A and N_A gradients in flush-type species increased during leaf development and were almost invariable through the rest of the season, while in continuously leaf-forming species, trait gradients increased constantly with increasing leaf age. In forbs, NA plasticity increased, while in grasses, NA plasticity decreased with increasing leaf age, reflecting life form differences in age-dependent changes in light availability and in nitrogen resorption for growth of generative organs. Although more work is needed to improve the coverage of age-dependent plasticity changes in some plant life forms, I argue that the agedependent variation in trait plasticity uncovered in this study is large enough to warrant incorporation in simulations of canopy photosynthesis through the growing period.

Keywords

dry mass per unit area;	light availability; nitroge	n content; ontogeny; p	hotosynthetic	capacity;
plasticity				

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Introduction

Plant canopies, either simple monotypic or more complex multi-species canopies, are characterized by major reductions in light availability from canopy top to bottom (Goudriaan 2016; Hikosaka 2005, 2014; Kitajima et al. 2005; Niinemets and Anten 2009). The strong within-canopy light gradients are further associated with conspicuous within-canopy variations in foliage structural, chemical and physiological traits. In particular, foliage nitrogen content per area (N_A) and foliage photosynthetic capacity per area (N_A) increase with increasing long-term light availability in the canopy in all plant functional types (Niinemets et al. 2015 for a review). Such a positive scaling of N_A with long-term light availability importantly enhances whole canopy (whole plant) photosynthesis compared with a canopy consisting of leaves with invariable N_A (Anten 2016; Anten and Bastiaans 2016; Field 1983; Gutschick and Wiegel 1988; Hikosaka et al. 2016; Niinemets 2016).

While the positive scaling of N_A and A_A with increasing light availability in the canopy is a uniform response, plant life forms differ in the extent to which variations in N_A , the product of leaf dry mass per unit area (M_A) and nitrogen content per dry mass (N_M) , and in A_A , the product of M_A and photosynthetic capacity per dry mass (A_M) are driven by light-dependent changes in morphological (M_A) , and chemical and physiological $(N_M \text{ and } A_M)$ traits. To gain insight into the controls of N_A and A_A , its is important to consider that species strongly differ in the rates of leaf turnover and canopy expansion. In fast-growing continuously expanding canopies, younger leaves at the top of the canopy shade the older leaves. This is an inherent feature of herbaceous canopies (Hikosaka 2005; Kikuzawa 2003; Oikawa et al. 2006), but several woody species in aseasonal and seasonal climates can also form leaves continuously through the growing season either due to indeterminate growth of first flush shoots, multiple flushing and sylleptic shoot formation (Cline and Harrington 2007; Scarascia-Mugnozza et al. 1999). Given the continuous extension of existing shoots and formation of sylleptic shoots preferably in higher light (Pieters et al. 1999; Powell and Vescio 1986; Weih 2009; Wu and Hinckley 2001), lower canopy leaves are older than upper canopy leaves in such woody canopies (Dickmann et al. 1990; Kikuzawa 2003; Vapaavuori et al. 1989; Vapaavuori and Vuorinen 1989). In contrast, in flush-type deciduous woody species all foliage, and in flush-type evergreen species all current year foliage is formed simultaneously.

Major changes occur in leaf structure, chemistry and physiological activity during foliage development and aging (Miyazawa et al. 1998; Miyazawa and Terashima 2001; Niinemets et al. 2012), while light availability, in turn, can modify the rate of leaf development and senescence (Frantz et al. 2000; Ono et al. 2001; Vos and van der Putten 2001; Yasumura et al. 2007). Thus, presence of or lack of interactions of leaf age and light availability within the canopy can drive differences in the mechanisms of within-canopy photosynthetic acclimation. In continuously expanding canopies, most foliage has developed in a similar light environment, and the within-canopy gradients in N_A and A_A primarily reflect gradients in leaf age and re-acclimation to shade or leaf senescence in shade (Niinemets et al. 2015; Pons 2016). Thus, leaves at different positions in the canopy are structurally similar with only moderate within-canopy variations in M_A if at all, while there are major changes in N_M and A_M , reflecting nitrogen reallocation from older leaves to younger leaves to the

expanding lateral and axial canopy positions (Anten et al. 1998; Hikosaka 1996; Niinemets et al. 2015; Pons 2016; Pons and Pearcy 1994; Vapaavuori et al. 1989; Vapaavuori and Vuorinen 1989; Weih 2009). In contrast, in flush-type species, variation in M_A mainly drives changes in N_A and A_A , and A_M and A_M change little or are invariable within the canopy, indicating that the within-canopy variation in N_A and A_A in these species reflects foliage adaptation to growth light environment (Niinemets et al. 2015).

Despite the basic mechanisms of within-canopy adaptation have been highlighted, past studies have mainly investigated within-canopy variations in leaf traits at some fixed moment of time with a few exceptions (Bertheloot et al. 2008; DeJong and Doyle 1985; Drouet and Bonhomme 1999; Niinemets et al. 2004a). Yet, leaf age structure of canopies varies through the growing period. In flush type species, leaf age continuously increases from budburst until leaf senescence. Despite continuous leaf turnover in fast-growing canopies, the average leaf age also increases with increasing the canopy age and time from the start of the growing season. In herbaceous canopies, the formation of new leaves stops after the onset of generative phase of development (Benbi 1994; Evers et al. 2010; Sadras et al. 2000). Analogously, in fast-growing woody canopies in seasonal climates, shoot growth typically slows down before the end of the growing season. This is relevant as leaves at different positions in the canopy can be exposed to vastly different light availabilities through their life-span due to seasonal changes in light availability, and shading that can occur due to early canopy expansion in flush-type deciduous and evergreen species, growth of new leaf flushes in evergreen flush-type species and continued canopy expansion in fastgrowing herbaceous and woody species.

The key question is what is the capacity of foliage of different age to acclimate to prevailing light conditions during their development and what is the capacity of fully-developed and aging leaves to re-acclimate to changed light environments. There is some evidence that within-canopy gradients in leaf traits in young developing and weakly differentiated leaves are less steep than the trait gradients in mature canopies (Kull et al. 1998; Niinemets et al. 2004a; Noormets et al. 1996). On the other hand, once the leaves are anatomically fullydifferentiated, anatomical constraints such as limited cell wall exposed surface area for spacing of additional chloroplasts can strongly constrain photosynthetic re-acclimation to modified light environment (Oguchi et al. 2005; Oguchi et al. 2006; Yamashita et al. 2002). In addition, initiation of leaf senescence leads to coordinated dismantling of foliage enzymatic apparatus, and retranslocation of foliage nitrogen, ultimately limiting the capacity for acclimation to changed light conditions (Hikosaka et al. 1993; Pons and Jordi 1998). Furthermore, leaf senescence can be triggered at different times in different canopy positions in dependence on species ecological strategy and rate of leaf turnover (Koike 1990; Koike et al. 1992). This evidence collectively suggests that leaf trait vs. light relationships vary through the canopy development and with leaf aging from budburst until senescence, with stronger effects expected for more dynamic fast-growing canopies.

Here a meta-analysis of within-canopy long-term light vs. leaf trait relationships across canopies of different age was carried out to gain insight into the dynamics of within-canopy trait variations in different plant life forms. The key aims of this analysis were: (1) to study how stable are leaf trait vs. light relationships in canopies with different leaf age, (2) to

characterize how leaf age effects on within-canopy variation in leaf traits differ among plant life forms with different rate of leaf turnover, and (3) to understand what developmental, structural and physiological factors control the age-dependent variations in leaf trait vs. light relationships in different plant life forms. It was hypothesized that the light-dependent plasticity (trait variation for a given variation in light availability within the canopy) of leaf traits first increases with increasing leaf age due to foliage morphological and physiological differentiation and thereafter decreases due to structural constraints on re-acclimation and induction of senescence, and that these changes are more profound in species with greater leaf turnover. Overall, this analysis highlights significant changes in within-canopy leaf trait responses in dependence on leaf age and plant life form and emphasizes that the dynamics of canopy development needs consideration in simulating whole canopy carbon gain. Definition of all symbols used is provided in Table 1.

Material and Methods

Dataset

The study consisted of a subset of the global database of within-canopy variation in leaf traits (Niinemets et al. 2015). In particular, the data analyzed here come from studies where within-canopy gradients have been investigated for the same canopies repeatedly at different time points, or in the case of evergreens, from studies where multiple leaf age classes had been studied at the same time. Altogether 71 canopies that provided data for 238 within-canopy gradients were included in the analysis (Table 2). These 71 individual canopies corresponded to canopies of different species, different environmental and spacing treatments for given species (fertilization, ozone, drought and sowing density treatments), and replicates in time (studies conducted with herbaceous or deciduous species in different years; Table 2). In the case of multi-species canopies, each species was considered individually (Table 2). For studies with treatments, the treatment effect per se was not analyzed here, but given that treatments typically resulted in significant differences in overall light penetration and light availability at different height levels in the canopy, each treatment is considered as an individual canopy replicate (Table 2).

In the previous study, within-canopy variation in 12 key leaf traits was analyzed (Niinemets et al. 2015). Due to limited number of observations for several traits, here I analyze age effects on within-canopy variation in leaf dry mass per unit area (M_A , available for 28 canopies), leaf nitrogen content per area (N_A , 45 canopies), leaf nitrogen content per dry mass (N_M , 20 canopies) and photosynthetic capacity (A_A , light-saturated net assimilation rate at ambient CO_2 concentration, 15 canopies).

Based on the number of gradients available, the species were grouped according to life form as needle-leaved evergreen trees (all conifer species), broad-leaved evergreen, broad-leaved winter deciduous trees, forbs and grasses. As explained below, winter-deciduous species were further divided between flush-type and continuous-type species based on the pattern of canopy development. In the case of grasses, all species were graminoid grasses, except *Carex acutiformis* that is a cyperoid grass with different pattern of leaf development. Graminoid grasses included in this analysis had a pronounced stem with apical growth where the youngest leaves are at the top of the stem. In the cyperoid perennial rhizomatous

grass *C. acutiformis*, the bulk of the plants in natural stands are vegetative and do not form a stem. Thus, long leaves extend from the base of the plant such that the upper leaf parts are the oldest. These differences in canopy formation were associated with contrasting age-dependent changes in trait plasticity as explained in the Results. To save space, data for most traits are plotted on the same figure panels for conifers and evergreen broad-leaved species and for forbs and grasses (herbs), but still maintaining the life form grouping.

Long-term integrated light availability

Given the evidence that within-canopy acclimation of leaf traits is driven by total integrated light rather than by relative light or instantaneous light (Chabot et al. 1979; Niinemets and Keenan 2012), this analysis is based on integrated quantum flux density (Q_{int}) that was estimated for each study in a consistent manner as explained in detail in Niinemets et al. (2015). In that study, Q_{int} was defined as a standardized estimate of light availability that corresponds to daily integrated quantum flux density averaged for 50 days after the start of foliage development, or to daily integrated quantum flux density averaged for the actual number of days since the start of foliage development when leaves were either younger than 50 days or when the average leaf life-span for the given species was less than 50 days $(Q_{\text{int.b50}})$; (Niinemets et al. 2015). This time period reflects the evidence from past studies indicating that foliage trait vs. integrated average light dependencies stabilize 30-60 d after leaf formation in canopies of both woody and herbaceous species (Niinemets and Keenan 2012; Niinemets et al. 2004a). However, given that canopies of widely varying leaf age were considered in this analysis, and that there can be a certain re-acclimation to current light availability once the leaves have fully matured, several additional estimates of long-term light availability were estimated according to the methodology of Niinemets and Keenan (2012): Q_{int} averaged for 30 days since the start of foliage development or for the actual number of days since the onset of leaf development when leaves were younger than 30 days $(Q_{\text{int,b30}})$, Q_{int} averaged analogously for 60 $(Q_{\text{int,b60}})$ days since the start of foliage development, and $Q_{\rm int}$ averaged for 50 days preceding leaf sampling or actual number of days when leaves were younger than 50 days ($Q_{\text{int,p50}}$).

In evergreens supporting foliage formed in different years, the estimates $Q_{\rm int,b30}$, $Q_{\rm int,b50}$ and $Q_{\rm int,b60}$ refer to the year of formation of foliage, but estimation of actual light availability during formation of older leaves is difficult due to time-dependent increases in self-shading (Brooks et al. 1994; Brooks et al. 1996; Niinemets et al. 2006a). For studies with evergreens where multiple leaf ages were studied at the same time, $Q_{\rm int,b50}$ values corresponding to the formation of the most recent leaf flush ($Q_{\rm int,Rb50}$) were also estimated for older leaf classes. These latter estimates rather than $Q_{\rm int,b50}$ have been conventionally used in studies looking at within-canopy trait variations in evergreens (e.g., Warren and Adams 2001; Wright et al. 2006). They do not reflect the light availability during foliage formation, but are also not biased by possible underestimation of growth light environment due to enhanced self-shading during new foliage growth. Across all the data, the above-canopy estimates, $Q_{\rm int,b50}$, $Q_{\rm int,b50}$ and $Q_{\rm int,b60}$ were strongly correlated (for linear regressions, $r^2 = 0.87 - 0.97$, P < 0.001 for all), but the correlations among $Q_{\rm int,b50}$ and $Q_{\rm int,b50}$

(spring, summer vs. autumn) in three subtropical conifers, *Pinus palustris*, *P. taeda* and *P. virginiana* (Table 2). In the case of $Q_{\rm int,b50}$ and $Q_{\rm int,p50}$, differences in light estimates underscored the seasonal variation in solar radiation through canopy development. Despite this variability, leaf plasticity estimates (see next section) and plasticity vs. leaf age responses were similar for different light estimates. Thus, in the final analysis, the relationships based on only $Q_{\rm int,b50}$ are demonstrated. When pertinent, quantitative and qualitative differences with other light estimates are highlighted in the appropriate sections of the Results.

Although some leaf traits might even respond to average light availability shorter than 5-10 days (e.g., Blanch et al. 2011; Logan et al. 1998; Niinemets et al. 1998), $Q_{\rm int}$ values for very short time periods, less than 5-10 days cannot be sufficiently precisely estimated for a global analysis due to uncertainties with dates of the onset of leaf development and uncertainties in global radiation databases (Niinemets and Keenan 2012). Nevertheless, provided leaf phenological characteristics could be reliably worked out from the data (s. below for leaf age estimation), adequate periods of integration were used for leaves younger than 50 days as well.

Data fitting and estimation of relative trait plasticity and standardized relative plasticity

 $Q_{\rm int}$ vs. leaf trait data were fitted in all cases with three parameter non-linear equations (monomolecular equation for positive relationships and a modified monomolecular equation for negative relationships, Niinemets et al. 2015) that provided in all cases good fits to data (Fig. 1 for sample data fits for different-aged leaves of *Quercus ilex*) as explained in detail in Niinemets et al. (2015). For each leaf age class, the minimum $Q_{\rm int}$ value present ($Q_{\rm int,min}$) was also estimated to characterize the age-dependent changes in light availability under specific conditions of the study.

There are two major difficulties in directly comparing $Q_{\rm int}$ vs. leaf trait relationships among different traits and among different plant life forms. First, different functional traits have numerically different values and functional traits vary among plant functional types (Niinemets et al. 2015; Wright et al. 2004), implying that the absolute values of trait change inherently differ for different traits and different plant functional types. Second, $Q_{\rm int}$ vs. trait relationships typically are non-linear (Niinemets and Keenan 2012; Poorter et al. 2009), implying that the change in a trait value at a given change in $Q_{\rm int}$ (trait plasticity) varies over the $Q_{\rm int}$ gradient. Thus, conclusive comparisons of trait plasticity can be only made over the same light range. Yet, $Q_{\rm int}$ ranges available varied among the studies due to several constraints, e.g., in multi-species canopies, shade-intolerant species often do not support leaves at low light in multi-species canopies, while understory species may not have been exposed to high $Q_{\rm int}$ values, limiting the gradient range (Niinemets 2016; Niinemets et al. 2015). Both of these limitations were addressed by calculating the relative light-dependent plasticity ($P_{\rm L}$) of a given trait over the given $Q_{\rm int}$ range:

$$P_{\text{L,1}} = \frac{v_{\text{i+x}} - v_{\text{i}}}{\Delta Q_{\text{int}}(v_{\text{i+x}} + v_{\text{i}})/2}, \quad (1)$$

where v_i is the trait estimate corresponding to the seasonal average quantum flux density $Q_{\text{int,i}}$ and v_{i+x} is the trait estimate corresponding to the second higher Q_{int} value ($Q_{\text{int,i+x}}$), and Q_{in} is given as $Q_{\text{int,i+x}}$ - $Q_{\text{int,i}}$, and the subscript I denotes the light range. Normalization of the trait plasticity with respect to the average trait value over the given Q_{int} range, ($v_{i+x} + v_i$)/2 allows for direct comparison of plasticity of traits with different absolute values.

As defined here, the trait plasticity characterizes the trait variation corresponding to the given long-term variation in within-canopy light availability. This definition adheres to the traditional definition of phenotypic plasticity in studies investigating plant response to long-term light gradients (Portsmuth and Niinemets 2007; Valladares 2003; Valladares et al. 2007). Some plant traits can also respond to rapid changes in light availability within and among days (e.g., Niinemets 2016), but such rapid responses, trait changeability, cannot be singled out within the current dataset.

Given that leaf trait vs. $Q_{\rm int}$ relationships are non-linear, it would be mathematically more correct to describe the plasticity as the standardized slope at the specified $Q_{\rm int}$, but such an estimate can be overly sensitive to small uncertainties at the edges of available $Q_{\rm int}$ range where the non-linear fits become most uncertain (Niinemets et al. 2015). Although assuming a local linear response, the plasticity estimated by Eq. 1 is less sensitive to such uncertainties and over a sufficiently narrow $Q_{\rm int}$ range approximates well the true plasticity. Here, the relative light-dependent plasticity was calculated over the $Q_{\rm int}$ ranges 3-6, 6-12, 12-20, 20-30, and 30-40 mol m⁻² d⁻¹. In these calculations, the trait values, $v_{\rm i}$, were estimated from the parameterized trait vs. $Q_{\rm int}$ responses using the extrapolation constraints specified in Niinemets et al. (2015). As the ranking of $P_{\rm L,l}$ values for different traits and for different plant functional traits seldom changed for different light ranges used, and the light range 6-12 mol m⁻² d⁻¹ was available for the largest number of cases (Niinemets et al. 2015), this range was used to compare age-dependent changes in relative plasticity.

 $P_{\rm L,l}$ values for different traits typically vary among species and plant functional types and can be also affected by experimental treatment effects (e.g., Dreccer et al. 2000; Hirose et al. 1988). Such confounding effects can partly blur the leaf age effects on trait plasticity. To nail down the age effects per se, standardized relative plasticity was calculated as:

$$S_{j,1} = \frac{P_{L,1,j}}{\sum_{j=1}^{j=n} P_{L,1,j}},$$
(2)

where $P_{L,l,j}$ is the relative trait plasticity for the leaf age class j and n is the number of leaf age classes present. Average $S_{j,l}$ across all ages available for the given canopy is equal to 1. $S_{j,l}$ allows for analyses of broad age-dependent trends in the given trait through all the data without confounding effects of inherent plasticity differences among species and plant functional types, and it also allows for comparison of age-dependent trends among different traits. However, $S_{j,l}$ could be overly sensitive if $P_{L,l}$ values for the given canopy are small,

indicating low plasticity. As $P_{L,l}$ values for N_M in most tree species were very low, $S_{j,l}$ estimates were not calculated for N_M in trees.

Although the $Q_{\rm int}$ range 6-12 mol m⁻² d⁻¹ was the most frequently present, it was still missing for some canopy gradients. Because $P_{\rm L,l}$ depend on the light range I, averaging $P_{\rm L,l}$ values across light ranges is not meaningful. Differently from $P_{\rm L,l}$, $S_{\rm j,l}$ values are not dependent on light range and thus, $S_{\rm j,l}$ values can be averaged across light ranges even if some ranges are missing. Thus, in addition to $S_{\rm j,l}$ values for the $Q_{\rm int}$ range 6-12 mol m⁻² d⁻¹, average $S_{\rm j,l}$ values for all available light classes were calculated $(\bar{S}_{\rm j})$. This made it possible to consider all light gradients in the ultimate analysis of age effects on within-canopy trait plasticity.

Estimation of average leaf age

In the current study, the tree species available originated mainly from temperate and Mediterranean climates, while subtropics was represented by three conifers (*Pinus palustris*, P. taeda, P. virginiana) and the broad-leaved species Rhizophora stylosa and tropics by the broad-leaved species Mangifera indica. In the case of most deciduous and evergreen trees in seasonal climates with pronounced cold period during winter, budburst occurs in spring at almost the same time through the canopy. Thus, all foliage in deciduous species, and foliage corresponding to the current leaf flush in evergreens has the same age. Typically, only one leaf flush is formed in the given year in cool temperate climates, while multiple leaf flushes through the growing season can be produced in warmer climates (Niinemets 2014). In the case of evergreens in our study, multiple annual leaf flushes were present in three subtropical pines (P. palustris, P. taeda and P. virginiana; Table 2), and in broad-leaved evergreens Mangifera indica (Bally 2006) and Rhizophora stylosa (Sharma et al. 2010), but not in the other species. The Mediterranean evergreen species Quercus ilex can have multiple leaf flushes, but in this analysis, a northernmost population was included that only supports one annual leaf flush (Niinemets et al. 2006a). In the case of single-flush deciduous and evergreen and multiple-flush evergreen species, leaf age was estimated considering the date of leaf sampling and estimated budburst date as explained in Niinemets et al. (2015).

Differently from flush-type species, an almost continuous- or semi-continuous-type of leaf formation in seasonal climates can occur in fast-growing early-successional forest species such as *Salix* spp. or young *Alnus* spp., *Betula* spp., and *Populus* spp. (Cline and Harrington 2007), but also in several fast-growing woody crops such as some cultured *Prunus* spp. (e.g., Gordon et al. 2006; Kervella et al. 1995). Among the winter-deciduous woody species in our study, only the young canopies of *Betula platyphylla* (Hoshika et al. 2013; Terazawa and Kikuzawa 1994) and canopies of *Prunus persica* (Gordon et al. 2006; Kervella et al. 1995) were characterized by continuous- to semi-continuous-type of leaf formation. In contrast, in annual and perennial herbs forming each year a new canopy, there is always a gradient in leaf age from canopy top to bottom. Furthermore, in grasses with basipetal leaf development, there is a significant gradient of leaf age within single leaves. This inherent developmental gradient is characteristic of all fast-growing canopies. For multiple sampling events, the question is however, how does average leaf age (\bar{a}) change with increasing canopy age (a_C). The age of individual leaves in different positions in the canopy was

commonly not reported, but $\alpha_{\rm C}$ was available or could be derived based on the dates of canopy development and foliage harvesting. Due to the within-canopy leaf developmental gradient, canopy age significantly overestimates $\bar{\alpha}$ of the canopy. This is especially relevant if $\alpha_{\rm C}$ exceeds the leaf life span, implying that leaves in the lower canopy have started to die off with the expansion of the canopy from the top. Assuming that the phyllochron (ϕ , time elapsed between the formation of subsequent leaves on the main stem) is constant during canopy development until the completion of leaf formation (e.g., Davidson et al. 2015), $\bar{\alpha}$ for a young mature canopy that has just completed leaf formation is given as:

$$\overline{\alpha}_{\mathrm{F}} = \phi \frac{n_{\mathrm{L}} + 1}{2},$$
 (3)

where n_L is the total number of leaves in the canopy. Average leaf age of a canopy after elapsing a time of t, \bar{a}_{F+} t, would become $\bar{a}_{F}+$ t. However, after full canopy expansion, there is a gradual loss of leaves from the lower canopy (e.g., Archontoulis et al. 2011; Hirose and Oikawa 2012; Milroy et al. 2001; Miyaji 1984). Thus, in determining changes in canopy average leaf age, it is important to consider that the longevity of the oldest (commonly lowermost) leaves cannot exceed their potential longevity. Therefore, in the current study, \bar{a} corresponding to different canopy ages was found on the basis of the following considerations. Whenever ϕ was available or reported in the literature (e.g., Virdis et al. 2009; Yin and Kropff 1996), this estimate was used to determine \bar{a} . In most cases, ϕ was not available, and ϕ for fully-developed young canopies was estimated as a_C/n_L . Although ϕ may vary somewhat through canopy development and depend also on environmental conditions, the curvilinearity of leaf number vs. time relationship is generally moderate (Virdis et al. 2009; Yin and Kropff 1996). Thus, this estimate can be considered as a good approximation of ϕ for the canopy. For older canopies with age a_C+ t, the number of leaves younger than the leaf longevity λ , n_{L+} t, was estimated first and then \bar{a}_{E+} t as:

$$\overline{\alpha}_{\text{F}+\Delta t} = \phi \frac{n_{\text{L}+\Delta t} + 1}{2} + \Delta t \quad (4)$$

If original papers reported presence of senescent leaves, presence of senescent leaves was taken as an estimate of λ for the given species in the given study. However, this was seldom the case, and an extensive literature search was carried out to obtain estimates of λ , and ultimately, average values of λ were calculated across all studies providing estimates of λ for the given species. However, average leaf longevity is not constant through the canopy, tending to be greater at the bottom of the canopy than in the top (Miyaji 1984). Given that only species-specific average values of leaf longevity, $\bar{\lambda}$, were available, λ was approximated as $1.3\bar{\lambda}$. This approximation assured that \bar{a} of the oldest senescent canopies was close to $\bar{\lambda}$.

As the values of n_L for herbaceous canopies with multiple sampling events were rarely available in the original studies, species-specific estimates were derived from the literature. For canopies with multiple sampling events, the following values of n_L were used: *Cynara*

cardunculus – 57 (Virdis et al. 2009); Glycine max – 34 (Munger et al. 1987); Gossypium hirsutum – 17 (Alarcon and Sassenrath 2011; Patterson 1988); Helianthus annuus – 25 (Hernández 2010); Hibiscus cannabinus – 60 (Abdul-Hamid et al. 2009); Oryza sativa – 12 (Yin and Kropff 1996); Triticum aestivum – 22 (Bos and Neuteboom 1998); Vicia faba – 19 (Chbouki et al. 2005); Xanthium strumarium var. canadense – 15 (Hirose and Oikawa 2012; Regnier and Harrison 1993); Zea mays – 14 (Drouet and Bonhomme 1999; Ford et al. 2008; Padilla and Otegui 2005). In the case of two woody species with continuous to semicontinuous leaf formation, Betula platyphylla and Prunus persica, leaf number per shoot (n_S) is the equivalent of n_L in herbaceous species. In B. platyphylla, values of n_S = 15 and ϕ = of 6 d were used (Hoshika et al. 2013; Terazawa and Kikuzawa 1994). In P. persica, values of n_S = 50 and ϕ = 4 d were used (Kervella et al. 1995).

Given that across all the data, canopy age and average leaf age were significantly correlated ($r^2 = 0.64$, P < 0.001) and the statistical relationships among trait plasticity and α_C were weaker than with $\bar{\alpha}$, only the relationships with $\bar{\alpha}$ are demonstrated.

Data analysis

Linear and second order polynomial regression analyses were carried out to analyze the statistical relationships between leaf age and relative (Eq. 1) and standardized (Eq. 2-3) plasticity estimates and among plasticity estimates for different traits, and relationships of plasticity estimates with minimum quantum flux density using R version 3.1.1 (R Development Core Team 2012). The regression analyses were conducted separately within plant life forms and through all data pooled. To improve normality of data and regression residuals, leaf age was log-transformed in these analyses. All relationships were considered significant at P < 0.05.

Results

Changes in the minimum light availability through canopy development

As noted in the introduction, in evergreen species, older leaves are expected to be overtopped by younger leaves, and thus, it is predicted that the light availability incident to older leaves decreases with increasing leaf age. Although the light availability for leaves developed at higher light decreases, the other question is whether the older leaves could be supported at lower light availabilities than those available for the younger leaves, i.e. whether there is a reduction in minimum light availability ($Q_{\rm int,min}$) with increasing leaf age. Indeed, there was evidence of a reduction of $Q_{\rm int,min}$ with increasing leaf age in several conifer (e.g., A. amabilis, P. densiflora, Fig. 2a) and broad-leaved (Quercus glauca, Q. ilex) species. However, this was not always the case, and $Q_{\rm int,min}$ even increased in Mangifera indica (Fig. 2a).

In winter-deciduous species, $Q_{\rm int,min}$ initially increased with increasing leaf age in developing canopies (*Populus tremula* and *Tilia cordata* that had the youngest leaves present in this dataset, Fig. 2b), reflecting overall low integrated incident quantum flux densities in the spring when the days will still short. In flush-type species, $Q_{\rm int,min}$ was constant in mature non-senescent canopies until leaf senescence (Fig. 2b). In contrast, canopies with

continuous- to semi-continuous-type of leaf formation that produced leaves through the growing season had the minimum $Q_{\text{int,min}}$ values in mid-season, and $Q_{\text{int,min}}$ increased later in the season after the initiation of senescence of leaves formed in the beginning of the growing season (Fig. 2b).

In herbaceous species with continuous expansion of canopy until the onset of the generative phase of development, variation of $Q_{\rm int,min}$ with average leaf age differed among forbs and grasses (Fig. 2c, d). In forbs where senescent leaves are abscised, an initial reduction in $Q_{\rm int,min}$ observed in several species (e.g., *Helianthus annuus* and *Xanthium strumarium* var. *canadense*, *Hibiscus cannabinus*; Fig. 2c) was followed by an increase in $Q_{\rm int,min}$ in the bulk of the species, although the increase in $Q_{\rm int,min}$ started at different times in different species (Fig. 2c). In contrast, in grasses where senescent leaves remain attached to the plant and can potentially shade physiologically-active neighboring leaves, $Q_{\rm int,min}$ decreased with increasing leaf age (Fig. 2d).

Age-dependent changes in leaf dry mass per unit area (M_A) and its light-dependent plasticity

In evergreens, M_A at a constant Q_{int} increased with increasing leaf age, except for the subtropical conifers *Pinus palustris*, *P. taeda*, and *P. virginiana* that produce multiple annual leaf flushes (Fig. 3a). In deciduous tree species, M_A initially increased with increasing leaf age until completion of leaf structural differentiation and was constant or moderately increased or decreased with further increases in leaf age similarly in both flush- and continuous-type species (Fig. 3b). Limited data available for herbaceous species indicated an increase of M_A with increasing leaf age (Fig. 3c). In the cyperoid grass *Carex acutiformis*, M_A values stabilized with further increases in leaf age.

In evergreens, the explained variance of M_A vs. Q_{int} relationships (Fig. 1a for sample relationships) typically decreased with increasing leaf age with the exception of *Abies veitchii*, *Picea abies* (study of Koppel and Frey 1984), *Picea sitchensis* (both studies available), *P. taeda*, and *Pinus virginiana* (Fig. 3d). In most deciduous species, r^2 values initially increased until full leaf development and were thereafter constant for the rest of the growing season or somewhat decreased in the middle of the growing season (Fig. 3e). In the three forb species available, there was a negative age-dependent trend in r^2 , but not in the grass *C. acutiformis* (Fig. 3f). Despite the moderate age-dependent variation in r^2 in deciduous trees and in herbs, r^2 values were maintained at a high level with a few exceptions (Fig. 3e, f).

In general, the relative light-dependent plasticity of M_A (Eq. 1) over the $Q_{\rm int}$ range of 6-12 mol m⁻² d⁻¹ ($P_{\rm L,6-12}$) decreased with increasing leaf age in evergreens with the exception of the conifer *Picea abies* (study of Koppel and Frey 1984) and the broad-leaved evergreen *Mangifera indica* (Fig. 4a). Further analysis of the standardized relative plasticity (S_j) over the same light range ($S_{j,6-12}$) indicated that even after standardization, a certain species-specific variability was evident (Supplementary Information, Fig. S1a). Calculation of the average standardized relative plasticity values over all available light ranges (\bar{S}_j), allowed for inclusion of all canopies with available M_A vs. $Q_{\rm int}$ responses. Examination of \bar{S}_j values further underscored the broad trend of decreasing plasticity with increasing leaf age (Fig.

4d). Both $S_{j,6-12}$ and \bar{S}_j values indicated that age-dependent changes in M_A plasticity were relatively moderate, within ca. ± 0.5 relative units from the average of all leaf ages measured in the given canopy (Fig. 4d, Supplementary Information, Fig. S1a).

In deciduous species, significant differences were observed in age-dependent changes in $M_{\rm A}$ plasticity among flush- and continuous-type species. In flush-type species, the plasticity estimates increased in the youngest leaves and were thereafter almost invariable during the rest of the growing season (Fig. 4b, e). In continuous-type species, the plasticity estimates monotonically increased from leaf development until senescence (Fig. 4b, e). Comparison of standardized plasticity estimates $S_{\rm j,6-12}$ (Supplementary Information, Fig. S1b) and $\bar{S_{\rm j}}$ (Fig. 4e) demonstrated that the age effects on $M_{\rm A}$ plasticity were stronger in continuous- than in flush-type species.

In the case of herbaceous species with overall low M_A plasticity, the age-dependent variation in $P_{L,6-12}$ was also low in most cases (Fig. 4c). Further examination of $S_{j,6-12}$ and \bar{S}_j responses indicated a reduction of plasticity in three species and an increase in *Cynara cardunculus* (Fig. 4f, Supplementary Information, Fig. S1c).

Variations in leaf nitrogen contents per area (N_A) and dry mass (N_M) and their light-dependent plasticity

In evergreen species, N_A at a given $Q_{\rm int}$ was relatively constant or even somewhat increased (broad-leaved species Q. ilex, conifers P. densiflora and P. sylvestris) or declined (broad-leaved Quercus glauca and Mangifera indica and conifer P. virginiana) with increasing leaf age (Fig. 5a). The explained variance of N_A vs. $Q_{\rm int}$ relationships generally decreased with increasing leaf age (Fig. 5e).

In deciduous species, N_A slightly decreased in the beginning of the growing season, and was constant over most of the season and declined at the end of the season in senescent leaves (Fig. 5b). The explained variance of N_A vs. Q_{int} relationships initially increased with leaf maturation and was thereafter almost constant through the rest of the growing season or somewhat declined in the middle of the season and for senescing leaves in some species (Fig. 5f).

In forbs, N_A generally decreased moderately after an initial increase, although in some species N_A somewhat increased (C. cardunculus) or was invariable ($Hibiscus\ cannabinus$) during the experimental period (Fig. 5c). The trend of an age-dependent decline in N_A was similar in grasses (Fig. 5d) with the exception of $Zea\ mays$ and fertilized treatments of $Triticum\ aestivum$ (Fig. 5d). No clear time-dependent trend in r^2 values of N_A vs. Q_{int} relationships was evident in herbaceous species with r^2 values sometimes increasing with leaf development (forb $Gossypium\ hirsutum\ in$ Fig. 5g and $grasses\ Z.\ mays$, higher density treatment, and T. aestivum fertilized treatments, Fig. 5h), and decreasing in older leaves (two $Helianthus\ annuus\ gradients\ in\ Fig. 5g\ and\ <math>T$. $aestivum\ in\ Fig. 5h$), but in most cases, the age-dependent variation was small (Fig. 5g, h).

In evergreens, all N_A plasticity estimates generally decreased with increasing leaf age (Fig. 6a, e, Supplementary Information, Fig. S2a) with some deviation from the general pattern

only observed for $\bar{S_j}$ in subtropical conifers (Fig. 6e). In deciduous species, the plasticity estimates increased in the growing leaves, and they were constant or only moderately changed during the rest of the growing season (Fig. 6b, f, Supplementary Information, Fig. S2b). Due to limited number of observations, no clearcut differences were evident among flush- and continuous-type species (Fig. 6b, f). In forbs, N_A plasticity estimates generally increased with increasing leaf age. Only H cannabinus with a moderate change through leaf aging and some H annuus gradients with reduced $\bar{S_j}$ in older leaves constituted exceptions to the general trend (Fig. 6c, g, Supplementary Information, Fig. S2c). In contrast, N_A plasticity estimates decreased in all graminoid grasses (Fig. 6d, h, Supplementary Information, Fig. S2d), and were moderately affected by leaf age in the cyperoid grass C. acutiformis (Fig. 6h).

As N_A is the product of nitrogen content per dry mass (N_M) and M_A , examination of age-dependent changes in N_M allows for gaining a mechanistic insight into differences in age-dependent changes in N_A and M_A and their light-dependent plasticity. In evergreens, N_M at a given Q_{int} moderately decreased or was almost constant (Fig. 7a). In deciduous species, N_M was very high right after the budburst and declined rapidly to an almost constant value that was maintained until the onset of leaf senescence (Fig. 7b). In herbaceous species, N_M decreased monotonically with increasing leaf age in all cases, but the response was less in C. acutiformis that was the only grass species with available N_M data (Fig. 7c). The light-dependent variation in N_M in woody species was low (see Niinemets et al. 2015), and the age-dependent changes in plasticity estimates were scattered (data not shown). In forbs, N_M plasticity increased with increasing leaf age in all cases except for H. cannabinus (Fig. 7d for \bar{S}_j , responses were analogous for $P_{L,6-12}$ and $S_{j,6-12}$). N_M plasticity for the cyperoid grass C. acutiformis did not change with leaf aging (Fig. 7d).

Changes in photosynthetic capacity per area (A_A) and its light-dependent plasticity

In evergreens, A_A at a given Q_{int} tended to decrease with increasing leaf age with the exception of the conifer P. densiflora and the broad-leaved evergreen M. indica (Fig. 8a). Explained variance of A_A vs. Q_{int} relationships was higher in younger than in older leaves, except for Q. ilex where r^2 for current year leaves was less than that for one-year-old leaves, and in P. densiflora where r^2 strongly increased over a time period from autumn to spring (Fig. 8b).

In deciduous and herbaceous species, only limited data of age effects on A_A light gradients were available. In deciduous species, A_A initially increased as leaves matured, and declined in senescing leaves (Fig. 8b). In grasses, age-dependent responses in A_A were variable, with A_A declining in only one study with *Oryza sativa* (Fig. 8c). No clear pattern in r^2 of A_A vs. Q_{int} relationships was evident for deciduous species and grasses (Fig. 8c, f).

The plasticity of $A_{\rm A}$ in evergreens initially tended to increase with increasing leaf age, and decline thereafter, or the plasticity monotonically decreased with increasing leaf age (*Abies amabilis, Pinus contorta*), although the variability was occasionally large (Fig. 9a, d, Supplementary Information, Fig. S1d). In deciduous species, the age-dependent change in plasticity was small, only ca. $\pm 20\%$ relative to the average plasticity, but the age range was also less than for the other traits. Due to limited data, age-dependent changes in $A_{\rm A}$

plasticity could not be separated among flush- and continuous-type species (Fig. 9b, e, Supplementary Information, Fig. S1e). In grasses, the responses were variable (Fig. 9c, f, Supplementary Information, Fig. S1f), but \bar{S}_{j} data suggested an initial increase of plasticity followed by a reduction in senescing leaves (Fig. 9f).

Correlative relationships among light-driven plasticity and leaf age across plant life forms

As the age-dependent changes in all three different plasticity estimates, relative plasticity $P_{L,6-12}$, its corresponding standardized plasticity $S_{j,6-12}$ and standardized plasticity averaged for all available light ranges, \bar{S}_j , were similar, and the largest number of observations was available for \bar{S}_j , here only the correlations with leaf age (log-transformed in all cases) are shown with this plasticity estimate except when pertinent. As before, the correlations refer to plasticity estimated with $Q_{\rm int,b50}$, and the responses with plasticity values estimated by other average integrated light measures are demonstrated when these alternative estimates provided additional insight into the patterns. In evergreens, M_A plasticity data of *Picea abies* (Fig. 4a, d) and N_A standardized plasticity for the youngest leaf age of *Pinus contorta* (Fig. 6e) were apparently outliers and the following analysis is conducted with and without these data. Analogously, the cyperoid grass C. acutiformis has a different growth pattern than all other grasses included in this analysis (see Methods), and thus, the plasticity relationships in grasses were analyzed with and without this species.

In evergreen conifers and broad-leaved species, the plasticity for M_A was not significantly related to leaf age when these plant life forms were considered separately, but a negative correlation was observed when conifers and broad-leaved species were pooled (Table 3). Furthermore, when the data were analyzed without the outlying observation for P. abies (Fig. 4d), M_A plasticity for conifers was negatively associated with leaf age (Table 3), and the relationships for all evergreens pooled was stronger (r= -0.49, P< 0.01). When M_A plasticity for evergreens was calculated with $Q_{\text{int,b50}}$ values corresponding to the formation of the most recent leaf flush ($Q_{\text{int,Rb50}}$), the correlations with leaf age were weaker (data not shown).

The plasticity for $M_{\rm A}$, was positively correlated with leaf age for all deciduous species pooled, and especially when flush- and continuous-type species were separately considered (Table 3). In continuous-type species, the response was even stronger (r = 0.79, P < 0.001) when the plasticity was estimated using $Q_{\rm int}$ values averaged for 50 days preceding leaf sampling or actual number of days when leaves were younger than 50 days ($Q_{\rm int,p50}$). However, in the case of flush-type species, $\bar{S}_{\rm j}$ estimated with $Q_{\rm int,p50}$ was not correlated with leaf age (r = 0.27, P > 0.1). $M_{\rm A}$ plasticity for available herb species analyzed together was not related to leaf age (Table 3).

The plasticity for N_A decreased with increasing leaf age in conifers and for all evergreens analyzed together (Table 3). Without the outlying observation for P contorta (Fig. 6e), the relationships were significantly stronger for conifers (Table 3) and all evergreens pooled (r= -0.67, P< 0.001). In fact, the non-standardized plasticity, $P_{L,6-12}$ with all values included scaled strongly with leaf age (r= -0.62, P< 0.02 for conifers and r= -0.57, P< 0.01 for all evergreens pooled), indicating that the standardization (Eq. 2) was overly sensitive in this particular case of P contorta. In deciduous species, N_A plasticity increased with leaf age in

flush-type species and when all deciduous species were pooled, but not in continuous-type species with limited data (Table 3). The relationship with flush-type species was statistically not significant when plasticity was calculated with $Q_{\text{int,p50}}$ (P > 0.07).

 $N_{\rm A}$ plasticity increased with leaf age in forbs, and tended to decrease in grasses (Table 3), especially without the outlying observations for *C. acutiformis* (Fig. 6h). Both these relationships were stronger when $\bar{S_j}$ was calculated with $Q_{\rm int,p50}$ (r= 0.39, P< 0.02 for forbs and r= -0.39, P< 0.04 for all grasses and r= -0.67, P< 0.001 without *C. acutiformis*) than with $Q_{\rm int,b50}$ (Table 3). In forbs and grasses, $N_{\rm A}$ plasticity changes were positively correlated with age-dependent changes in minimum light availability, but not in other plant life forms (Supplementary Information, Fig. S3).

The plasticity for $A_{\rm A}$ was positively associated with leaf age only in broad-leaved evergreens $(r=0.47,\,P<0.05)$ and not in any other species group (P>0.1). Analogously, $P_{\rm L,6-12}$ $(r=0.60,\,P<0.02)$ and $S_{\rm j,6-12}$ $(r=0.53,\,P<0.05)$ were positively correlated with leaf age in broad-leaved evergreens.

Controls of age-dependent plasticity change across different plant life forms

In woody species, $P_{L,6-12}$ values for N_A scaled positively with $P_{L,6-12}$ values for M_A across all leaf ages following a close to 1:1 relationship (Fig. 10a). In contrast, in herbaceous species, N_A plasticity was more strongly associated with N_M plasticity (Fig. 10b). Nevertheless, the data were positioned above the 1:1 line, indicating that M_A plasticity also played a role in age-dependent changes in N_A plasticity in herbaceous species (cf. Figs. 10a and 10b). Across all data pooled, A_A plasticity was correlated with N_A plasticity, but the relationship was weak (Fig. 10c).

Discussion

Why should light-dependent plasticity vary with canopy and leaf aging

A within-canopy gradient in leaf traits at some moment of time provides a snapshot of canopy functioning, and the key question is to what extent such a snapshot is representative for the entire growing season. As discussed in the introduction, leaf trait values at a certain within-canopy light availability can differ due to age-dependent changes in foliage structural and physiological differentiation as well as due to foliage re-acclimation to modified light conditions that can occur because of changes in light availability above the canopy during the season and within the canopy because of canopy expansion, resulting in shading of older leaves by newly developed leaves.

What is the evidence of dynamics in leaf age and light environment in canopies? Increases in average leaf age inevitably occur in flush-type woody species, but also in fast-growing continuously leaf-forming species due to cessation of canopy expansion and new leaf formation after the onset of generative phase of life cycle in herbs and changes in biomass allocation from production of new leaves to enhanced investment in woody tissues in fast-growing deciduous woody species. Indeed, significant changes in the minimum light availability ($Q_{\rm int,min}$) with leaf age were observed for a number of canopies (Fig. 2), although not always. As expected, $Q_{\rm int,min}$ decreased with increasing leaf age in several

evergreen canopies (Fig. 2a), but in some conifers, older leaves could not apparently be supported at increasingly low light intensities (Fig. 2a). This suggests a reduction of leaf survivorship once the shading by newer foliage becomes severe enough such that achieving a positive carbon balance becomes impossible (Hikosaka 2003; Hikosaka 2005; Niinemets 1997a). In deciduous woody species, $Q_{\rm int,min}$ initially increased due to increasing the daily integrated solar radiation in spring, but decreased thereafter to a constant level at full canopy expansion in flush-type woody species, and it was strongly reduced due to canopy expansion until the cessation of formation of new leaves in continuous-type woody species (Fig. 2b). As expected, $Q_{\rm int,min}$ typically decreased with increasing leaf age in grasses. However, in forbs (Fig. 2d), $Q_{\rm int,min}$ was surprisingly constant in most cases or even increased sometimes, suggesting that upon reaching a certain $Q_{\rm int,min}$, older leaves senesced and were abscised, although some species could support leaves at increasingly lower $Q_{\rm int,min}$ (Fig. 2d). This evidence altogether underscores that within-canopy light conditions do significantly vary through canopy development and leaf aging, providing a mechanistic basis for age-dependent changes in trait gradients.

Age-dependent changes in leaf structure and structural plasticity in different plant life forms

Structural leaf differentiation in developing leaves involves increases in overall leaf thickness, increases of individual mesophyll cells and thickness of cell walls (Hanba et al. 2001; Miyazawa et al. 1998; Miyazawa and Terashima 2001; Niinemets et al. 2012; Tosens et al. 2012), ultimately leading to increases in leaf dry mass per unit area (M_A) as was also observed in this meta-analysis (Fig. 3). Furthermore, in evergreen species, M_A of fully-developed leaves (Fig. 3a) changes with increasing leaf age due to secondary needle growth, i.e. growth of new xylem and phloem inside the needle central cylinder (Ewers 1982; Gilmore et al. 1995).

Despite similar increases in M_A in different plant life forms, this meta-analysis highlighted profound differences in how M_A light-dependent plasticity depended on leaf age in different plant life forms. In evergreens, the strength (r^2) of average daily integrated light vs. Q_{int} relationships generally decreased (Fig. 3d) and the relative trait plasticity (P₁, Eq. 1, Fig. 4a) and standardized plasticity (S_i , Eq. 2; Fig. 4d and Table 3) decreased with increasing leaf age with the exception of the conifer *Picea abies*. Greater scatter of M_A vs. light responses and lower plasticity of older leaves in evergreens indicates that leaves developed at higher light cannot morphologically acclimate to reduced light conditions upon shading. Different plasticity response in *P. abies* remains unclear. This contrasting response might reflect the circumstance that within the conifer shoot, there is a large self-shading of adjacent needles, especially for high-light grown shoots (Cescatti and Zorer 2003; Niinemets et al. 2006b) such that leaf morphology varies in dependence on needle axial location (lateral, upper, lower) within the shoot (Norman and Jarvis 1974). Given that M_A (Fig. 3a) and $Q_{\text{int.min}}$ (Fig. 2a) values of *P. abies* were only moderately affected by leaf age, shading could have resulted in preferential abscission of needles less acclimated to the new lower light environment, leading to a virtual constancy of M_A values and plasticity.

Differently from evergreens, M_A plasticity increased with increasing leaf age in deciduous woody species (Fig. 4b, e and Table 3), whereas the increase was stronger for continuous-than for flush-type species. In fact, in flush-type species, the change in M_A plasticity occurred in early season during leaf development and the within-canopy M_A gradients were invariable for the rest of the growing season (Fig. 4b, e). In continuous-type species, the overall period for leaf development was longer due to new leaf formation, but clearly, as more leaves in the canopy reached the full maturity and became structurally differentiated, the structural gradient through the canopy increased. Greater capacity for changes in leaf structural characteristics in continuous-type species is also underscored by the stronger relationships when plasticity was calculated with $Q_{\rm int}$ values estimated as an average of 50 days before foliage collection ($Q_{\rm int,P50}$) rather than with $Q_{\rm int}$ values for 50 days since the budburst ($Q_{\rm int,b50}$).

A certain reduction in r^2 of M_A vs. $Q_{\rm int}$ relationships in the middle of growing season observed in several deciduous woody species (Fig. 3e) is surprising, but it can reflect $Q_{\rm int}$ -dependent differences in the accumulation of starch and soluble carbohydrates in leaves photosynthesizing most actively (Le Roux et al. 1999; Niinemets and Kull 1998). In the case of continuous-type deciduous woody species, it can also indicate formation of news leaves with initially weaker response to $Q_{\rm int}$ (Fig. 2b, 4e).

Analogously to continuous-type deciduous woody species, one would expect that in herbs, $M_{\rm A}$ plasticity increases with increasing the average leaf age as more leaves fully mature. However, herbs have a faster rate of leaf development than woody species (Miyazawa et al. 1998; Niinemets et al. 2012), implying that leaves developing under high light and becoming shaded with canopy expansion spend a shorter part of their whole developmental period in shade. This likely explains why $M_{\rm A}$ plasticity in herbs is typically lower than in woody species (Niinemets et al. 2015) as was also observed in our study (Fig. 3c). The plasticity responses observed within this narrow range of variation were contradictory (Fig. 4c, f), calling for further studies on age-dependent changes in morphological plasticity of herbs.

Age-dependent variations in nitrogen gradients differ among plant life forms

Leaf age effects on nitrogen content per area (N_A), the product of M_A and nitrogen content per dry mass (N_M) depend on variations in its components. Analogously, age effects on light-dependent plasticity of N_A are driven by the age effects on the plasticities of M_A and N_M . In evergreens, N_A was moderately affected by leaf age or even increased in some species (Fig. 5a), reflecting minor reductions in N_M (Fig. 7a) and increases in M_A (Fig. 3a) with increasing leaf age. A low age-dependent change in N_A with leaf age in non-senescent leaves of evergreens is in agreement with past observations (Cordell et al. 2001; Escudero and Mediavilla 2003; Rapp et al. 1999). Differently from evergreens, in deciduous woody species, N_A (Fig. 5b) decreased in the beginning of the growing season, was almost constant through most of the growing season and decreased after the onset of leaf senescence. Thus, even M_A increased in the growing leaves (Fig. 3b), age-dependent reduction in N_M was much stronger during early leaf development, while resorption of nitrogen from senescing leaves dominated the late-season reduction in N_A (Fig. 7b). Although N_M consistently decreased in forbs (Fig. 7c), N_A changed less in most cases (Fig. 5c), suggesting a

compensation due to increased M_A (Fig. 3c). In the case of grasses, limited data were available for N_M (Fig. 7c), but N_A decreased across available data as expected with some exceptions in fertilized treatments (Fig. 5d).

Given that in woody species, the within-canopy gradients in $N_{\rm M}$ were small (data not shown, see Niinemets et al. 2015), and variation in $N_{\rm A}$ plasticity was strongly associated with variation in $M_{\rm A}$ plasticity across species and leaf ages (Fig. 10a), the age effects on $N_{\rm A}$ plasticity were expected to mirror changes in $M_{\rm A}$ gradients. Indeed, in woody evergreens, both $N_{\rm A}$ plasticity (Fig. 6a, e and Table 3), and r^2 values of $N_{\rm A}$ vs. $Q_{\rm int}$ relationships (Fig. 5e) decreased with increasing leaf age similarly to corresponding $M_{\rm A}$ traits (cf. Fig. 3d, Fig. 4a, d and Table 3). Analogously to $M_{\rm A}$ plasticity, in deciduous woody species, $N_{\rm A}$ plasticity initially increased with increasing leaf age, reflecting early foliage morphological differentiation, and the plasticity changed thereafter little during the rest of the growing season (Fig. 6b, f and Table 3). While clear differences among flush-type and continuous-type species were observed for $M_{\rm A}$ plasticity, limited coverage of continuous-type species does not allow for conclusive comparisons of $N_{\rm A}$ plasticity among canopies with different leaf turnover.

In herbs, surprising differences were observed in age-dependent changes in N_A plasticity among forbs (Fig. 6c, g and Table 3) and grasses (Fig. 6d, h and Table 3). A moderate age-dependent increase in N_A plasticity in forbs was associated with limited age-dependent changes in N_A (Fig. 5c), major reductions in N_M (Fig. 7c) and increased N_M plasticity (Fig. 7d), and increased minimum light availability (Fig. 2c). In contrast, the age-dependent reduction in N_A plasticity in grasses was associated with reduced minimum light availability (Fig. 2d), while little data were available for age effects on other drivers except for the cyperoid grass *Carex acutiformis* that was an outlier in the grass N_A plasticity relationships (Fig. 6h). However, contrary to the graminoid grasses included in the analysis, the leaves of this species grow from the plant base such that the youngest foliage elements are at the bottom of the canopy, explaining the diverse response.

Despite incomplete evidence, one can still speculate about the possible mechanisms that is operative in age-dependent changes in N_A plasticity in herbaceous species. In forbs, the gradient becomes stronger as plants loose older leaves at the bottom of the canopy and recycle nitrogen from remaining lower leaves to the upper leaves (Fig. 7c), while upper leaves mature increasing their M_A and N_A values similarly to continuous-type flush species. In fact, although N_A plasticity across species and leaf ages in forbs was primarily driven by N_M plasticity (Fig. 10b), the data did not fall on a 1:1 line, suggesting a role in M_A plasticity as well (marginally significant in Fig. 10a). In grasses, much of the nitrogen seems to be resorbed from the upper canopy in the generative phase of leaf development for the growth of generative organs, while resorption from lower canopy leaves contributes less to generative growth. This suggestion is supported by evidence from classical studies on grasses, indicating that the uppermost flag leaves play a major role in providing nitrogen and carbohydrates to generative organs during grain filling (Araus et al. 1986; Evans 1983; Hunt 1985).

Changes in photosynthetic plasticity through leaf ages

Although the light-saturated net assimilation rate at current ambient CO₂ concentration, the photosynthetic capacity, per unit leaf area (A_A) typically scales with N_A (Evans 1989), A_A also depends on nitrogen use efficiency that characterizes the fraction of nitrogen invested in light-limiting components of photosynthesis machinery (e.g., Niinemets et al. 2015; Reich et al. 1995). In addition, in stressed leaves, photosynthetic capacity can be reduced due to reductions in stomatal conductance, inhibition of the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and photoinhibition (e.g., Flexas et al. 2004; Werner et al. 2001). In the current study, age-dependent changes in A_A at a given $Q_{\rm int}$, a reduction with increasing leaf age in evergreens (Fig. 8a), and an increase in A_A during leaf development and reduction in senescing leaves in deciduous woody (Fig. 8b) and herbaceous (Fig. 8c) species broadly agreed with past observations (Niinemets et al. 2012 for a review). However, there were several exceptions such as age-dependent increases in evergreens Mangifera indica and Pinus densiflora (Fig. 8a) and a certain variability in herbs (Fig. 8c). Such discrepancies can be indicative of other leaf-age independent effects such as different level of stress, including photoinhibition, sustained through the autumn to spring season (Slot et al. 2005) and resorption and refilling of N pools in older leaves during growth of new spring leaf flush (Weikert et al. 1989) in P. densiflora as well as changes in source-sink relationships through the fruiting period in M. indica that can affect the activity of ratelimiting photosynthetic enzymes (Crafts-Brandner et al. 1991; Urban et al. 2004) in addition to alterations in nitrogen remobilization (Weinbaum et al. 1994).

Provided the changes in N_A drive within-canopy modifications in A_A , age-dependent changes in A_A plasticity should follow the age effects on N_A plasticity. This suggestion is confirmed by a correlation, although scattered, among A_A and N_A plasticities across all data (Fig. 10c). However, the data on A_A plasticity were limited, and within plant life forms, the trends in A_A plasticity were somewhat contradictory to N_A plasticity data. In fact, in broadleaved Rhizophora stylosa (Fig. 9a, d), AA plasticity increased with increasing leaf age reflecting mainly the increase through canopy development, consistent with the delayed photosynthetic development in evergreens (Miyazawa et al. 1998; Miyazawa and Terashima 2001). Analogously, greater plasticity in one-year-old leaves in *Quercus ilex* and *M. indica* may indicate delayed developmental effects. Furthermore, the reduction in A_A plasticity with leaf age in conifers was less than that in M_A and N_A plasticities (cf. Fig. 4a, d, Fig. 6a, e, Fig. 9a, d and Supplementary information Fig. S1 and S2). Despite the structural constraints on re-acclimation upon shading (s. Introduction), there is evidence of reacclimation to modified light conditions in conifers and broad-leaved evergreens through changes in nitrogen partitioning such that the photosynthetic plasticity of older leaves might remain relatively high despite reductions in the plasticities of M_A and N_A (Brooks et al. 1994; Brooks et al. 1996; Niinemets et al. 2006a). Nevertheless, the A_A vs. Q_{int} relationships became more scattered (Fig. 8d) and ultimately plasticity decreased, suggesting that the reacclimation is limited.

In broad-leaved species, the overall change in photosynthetic plasticity with leaf age was small (Fig. 9b, e), but the leaf age range was also less than for M_A and N_A , and obviously, the leaf early development and senescence periods were largely missing (cf. Fig. 4b, e and

Fig. 6b, f). However, differently from N_A plasticity, there was a certain reduction in A_A plasticity in the middle of the growing season. Such a reduction might be indicative of a stronger photosynthetic reduction due to reduced stomatal conductance in the upper canopy where the evaporative demand is greater, especially under soil drought conditions (Aasamaa et al. 2004; Niinemets et al. 2004b). A greater degree of reduction of photosynthesis rate due to photoinhibition in the upper canopy (Niinemets and Kull 2001; Werner et al. 2001) can also explain such a decline.

As in evergreens, photosynthesis in some deciduous woody species can re-acclimate, although slowly, to modified light conditions by changes in the size and number of chloroplasts per leaf area (Oguchi et al. 2005; Oguchi et al. 2006). In particular in continuous-type species where light availability strongly decreased (Fig. 2b) and $M_{\rm A}$ plasticity increased (Table 3) with increasing leaf age, $A_{\rm A}$ plasticity is expected to be changed as well, but there are currently no data to test this hypothesis.

In grasses, the initial increase in A_A plasticity during canopy development was followed by a reduction during grain filling period (Fig. 9c, f). This early increase suggests a somewhat longer developmental period for differentiation of photosynthetic apparatus, especially at higher light, than the developmental period for N_A (cf. Fig. 6h). The reduction of A_A plasticity later into the growing season is fully compatible with changes in N_A plasticity as nitrogen is resorbed from upper canopy leaves during grain filling (cf. Fig. 6h).

Limitations and potentials of the current analysis and a conspectus for the future work

Current study summarizes the bulk of the information on age-dependent changes in within-canopy gradients in leaf traits. Rigorous normalization procedures (Eq. 1-2) were developed to remove the interfering variation due to differences in absolute trait values among species, plant life forms, treatments and growth conditions. In addition, different integrated light values were employed to test for the effects of growth and current leaf light environment on trait plasticity changes.

Despite the compiled set of data constitutes the most extensive database on temporal variations in within-canopy trait gradients, this analysis highlights several key limitations with the existing body of data. First, in most studies, the age range investigated did not cover the whole leaf and canopy development from leaf initiation until senescence. In particular, there is a lack of data for within-canopy gradients in early season in woody species and for young developing canopies in herbaceous species. Analogously, there is a lack of data for late-season gradients. While these periods have not been in the focus of researchers due to overall low leaf physiological activities during these periods, having data over the entire growing season is fundamentally important for conclusive understanding of canopy developmental processes. Furthermore, leaf growth and senescence periods comprise a significant period of leaf life span, and for accurate simulation of canopy photosynthesis over the entire growing period, these parts of leaf life cannot be left out. Fore example, as the result of limited age range, early developmental responses in plasticity of all traits remain largely unknown for herbaceous species.

The second limitations is that only a few studies have looked at a suite of relevant functional traits simultaneously. This complicates drawing conclusions about the structural, chemical and physiological coordination of canopy functioning through the leaf age sequence. Particularly limited were data on photosynthesis, which is the key trait characterizing foliage physiological activity, but clearly a trait that is more complicated to measure than M_A and N_A . Finally, uneven coverage of traits for different life forms, e.g. limited coverage of M_A in herbs, and N_A and A_A for flush-type deciduous woody species, also complicates comparisons of age-dependent plasticity changes among plant life forms.

Lack of appropriate replication in several cases makes it difficult to distinguish among exceptional and characteristic responses, and thus, it limits the generality of some of the conclusions that can be drawn on the basis of these data. Exceptional, outlying responses, constitute an integral part of biological variability, and in the case of age effects on within-canopy plasticity of leaf traits, they might reflect multiple superimposed processes such as fruiting, and temporal variability in environmental and/or biological stresses. Future studies are needed to fill the outlined gaps in data coverage. Particular emphasis is needed on gaining a better insight into age-dependent variations in photosynthesis gradients through the canopies and on understanding the structural, chemical and physiological controls on development and re-acclimation of A_A gradients and appropriate time-kinetics in different plant life forms. Thus, while trying to understand the controls on temporal variations in within-canopy gradients in photosynthesis, it is relevant to consider all key functional traits, M_A , N_A and A_A simultaneously, and document the temporal variations in light environment above and within the canopy through the study period.

Conclusions and implications for modeling canopy photosynthesis

Major changes occur in leaf structure, chemistry and physiological activity during foliage development and aging, and as this study demonstrates, these age-dependent changes importantly modify the within-canopy variation in key foliage traits. The age effects differ among plant functional types and this diversity reflects fundamental differences in the rate of leaf turnover and in the duration of leaf developmental and senescence periods. In woody evergreens, plasticities in leaf dry mass (M_A) and nitrogen content (N_A) per area decrease with increasing leaf age, indicating limited re-acclimation capacity to modified light conditions, but there is a certain re-acclimation capacity in photosynthetic capacity per area (A_A) . In flush-type deciduous species, the plasticity changes mainly occur during early leaf development, while in continuous-type deciduous woody species and in herbaceous species, the plasticity changes occur through leaf life span. Significant differences, the increase in plasticity in continuous-type deciduous woody species and forbs and the reduction in grasses, are observed among species with fast leaf turnover as well, but the data coverage is not complete for all traits, limiting the generality of conclusions.

Currently, the age-dependent variation in foliage plasticity is largely ignored in simulations of canopy photosynthesis. In fact, in species with short-lived leaves, modeling studies currently typically use snapshots of canopy leaf trait gradients measured at a certain moment of time. In evergreens, studies either use current-year or one-year-old leaves, or some studies have pooled different leaf ages in developing N_A vs. Q_{int} and A_A vs. Q_{int} relationships

(Medhurst and Beadle 2005; Warren and Adams 2001; Wright et al. 2006). Although more experimental work is needed to improve the coverage of periods of leaf development and senescence in several plant life forms, the data available indicated that the age-dependent plasticity changes were typically within $\pm 50\%$ of average plasticity for the given canopies, and greater changes, occasionally more than $\pm 100\%$ were observed for some traits in several plant life forms. I argue that these changes need to be included in quantitative simulations of canopy photosynthesis through the whole growing period with major improvements expected for evergreens and for early and late growing season for all other plant life forms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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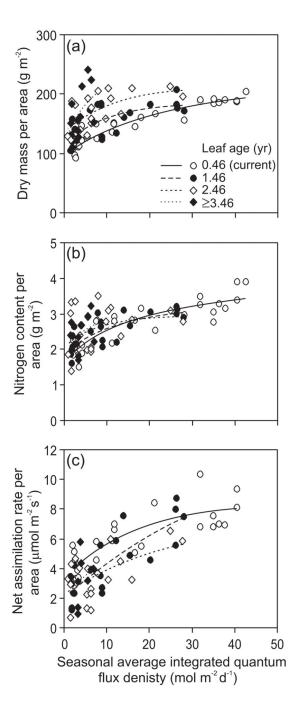


Fig. 1. Illustration of age-dependent changes in leaf dry mass per unit area (a), nitrogen content per unit area (b) and light-saturated net assimilation rate per unit area (c) for four leaf age classes in the Mediterranean evergreen broad-leaved species *Quercus ilex* (data of Niinemets et al. 2006a). Leaf age of 0.46 yr denotes current-year leaves. The data were fitted by a monomolecular asymptotic function in the form $y = y_{\text{max}}(1-e^{d-fx})$, where y_{max} , d and f are empirical coefficients. The three-parameter monomolecular function provides a better fit to the data (greater r^2) than two-parameter logarithmic or power functions typically used in

fitting non-linear asymptotic biological relationships (Niinemets and Keenan 2012; Niinemets et al. 2015).

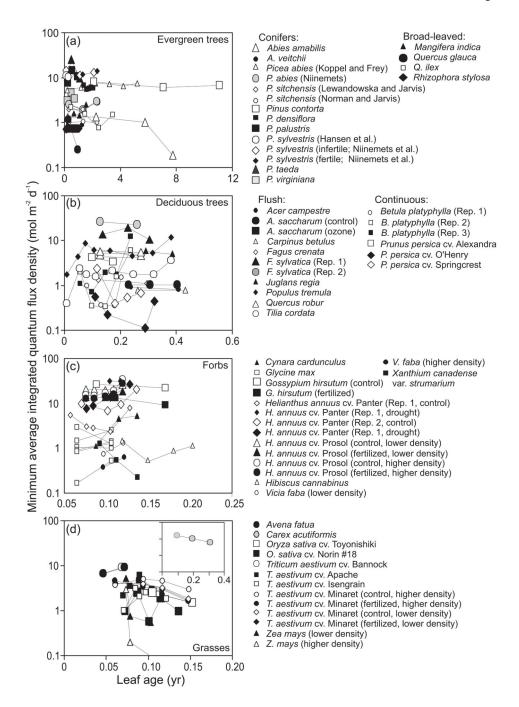


Fig. 2. Changes in the minimum light availability incident to the leaves ($Q_{\rm int,min}$) with increasing leaf age for evergreen conifers and evergreen broad-leaved trees (a), winter-deciduous trees (b), forbs (c) and grasses (d) for all canopies included in the analysis (Table 2 for details). The lines connect successive values of $Q_{\rm int,min}$ to better demonstrate time-dependent trends for the canopies included in the analysis. Light availability was characterized by daily integrated quantum flux density averaged for 50 days since leaf formation or for actual number of days when leaf age was less than 50 days ($Q_{\rm int}$, Material and methods and Table 1

for further information). In the case of deciduous species, herbs and forbs and in the evergreen broad-leaved species *Rhizophora stylosa* (Suwa and Hagihara 2008) and *Mangifera indica* (Urban et al. 2003) and in the evergreen conifer *Pinus sylvestris* in the study of Hansen et al. (2002), the same canopies were sampled at different moments of time. In the case of all other evergreen species, different-aged leaves were studied at the same time. Winter-deciduous species are separated among flush-type (all leaves formed at the same time in the beginning of the growing season) and continuous- to semi-continuous-type (leaves formed continuously or in multiple flushes on proleptic shoots, and on sylleptic shoots initiated through the growing season) species.

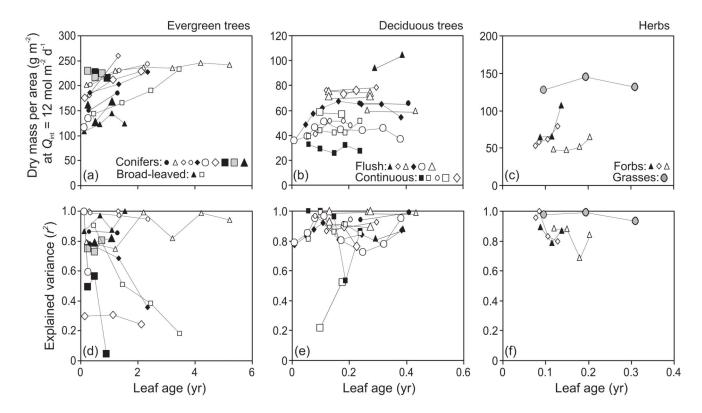


Fig. 3. Age-dependent changes in leaf dry mass per unit area (M_A) at a constant moderately high average daily integrated quantum flux density (Q_{int}) of 12 mol m⁻² d⁻¹ (a-c) and the degree of explained variance (r^2) of Q_{int} vs. M_A relationships fitted by the monomolecular function (d-f) in different plant life forms. Succeeding trait values for given canopies are connected by lines to highlight the temporal trends. Values of M_A at the given Q_{int} were derived from fitted Q_{int} vs. M_A relationships (Fig. 1a for sample data fits). Symbols and definition of Q_{int} as in Fig. 2.

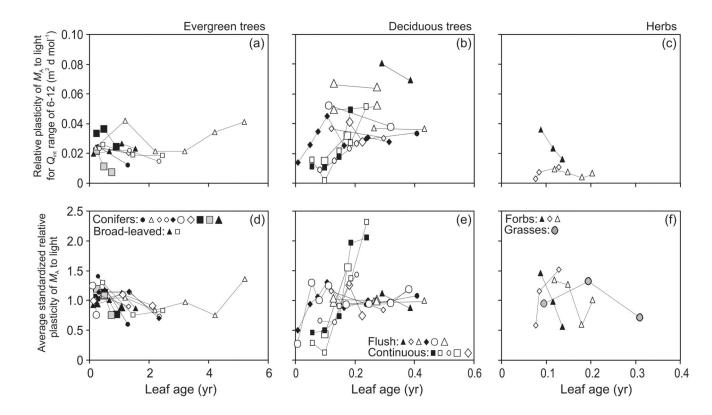


Fig. 4. Relative light-dependent plasticity (Eq. 1) of leaf dry mass per unit area ($M_{\rm A}$) over the integrated quantum flux density ($Q_{\rm int}$) range of 6-12 mol m⁻² d⁻¹ (a-c) and average standardized plasticity (Eq. 2) calculated over all available light ranges [(d-)(f); Material and methods for the light ranges used] in relation to leaf age in different plant life forms. The relative plasticity is the relative light-dependent change in the trait value normalized with respect to the light range. Thus, it allows for comparison of plasticities of given trait among species and plant functional types with different trait values at the given light level (Fig. 3a-c), and comparison of light-dependent changes in different traits for given species (Niinemets et al. 2015). The standardized relative plasticity further normalizes for the variation of relative plasticity among species, among plant functional types and among $Q_{\rm int}$ ranges. Symbols as in Fig. 2 and data presentation as in Fig. 3. Figure S1a-c shows standardized relative plasticity estimates corresponding to the $Q_{\rm int}$ range 6-12 mol m⁻² d⁻¹.

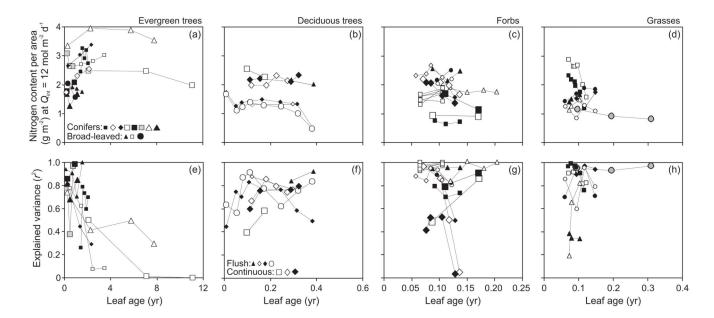


Fig. 5. Age-dependent modifications in leaf nitrogen content per unit area (N_A) at a constant average daily integrated quantum flux density (Q_{int}) of 12 mol m⁻² d⁻¹ (a-d) and the degree of explained variance (r^2) of Q_{int} vs. N_A relationships (e-h) in different plant life forms. Figure 1b demonstrates sample data fits. Symbols are defined in Fig. 2 and data presentation follows Fig. 3.

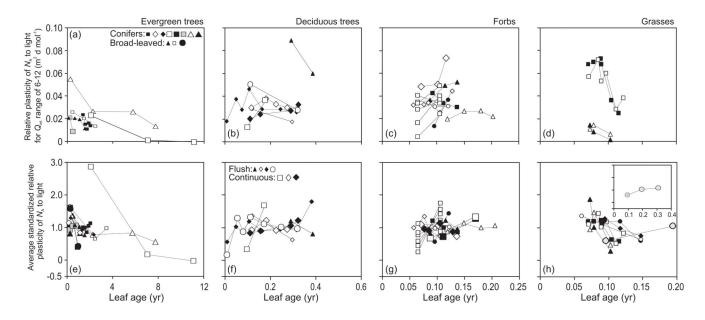


Fig. 6. Light-dependent plasticity (Eq. 1) of leaf nitrogen content per unit area (N_A) over the integrated quantum flux density (Q_{int}) range of 6-12 mol m⁻² d⁻¹ (a-d) and average standardized plasticity (Eq. 2) calculated over all available light ranges (e-h) in dependence on leaf age in different plant life forms. Symbols as in Fig. 2, data presentation as in Fig. 3 and definition of plasticity estimates as in Fig. 4. Figure S2 demonstrates changes in standardized relative plasticity (Eq. 2) over the Q_{int} range of 6-12 mol m⁻² d⁻¹.

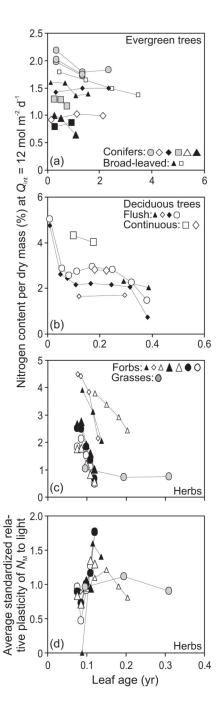


Fig. 7. Leaf age effects on leaf nitrogen content per dry mass ($N_{\rm M}$) at a constant average daily integrated quantum flux density ($Q_{\rm int}$) of 12 mol m⁻² d⁻¹ in different plant life forms (a-c), and average standardized plasticity to light (Eq. 2, Fig. 4 for plasticity definition) in herbs (d). Plasticity of $N_{\rm M}$ is only demonstrated for herbs because $N_{\rm M}$ was weakly related to $Q_{\rm int}$ in most tree species studied here in accordance with a global within-canopy plasticity dataset (Niinemets et al. 2015). Symbol definitions as in Fig. 2 and data presentation as in Fig. 3.

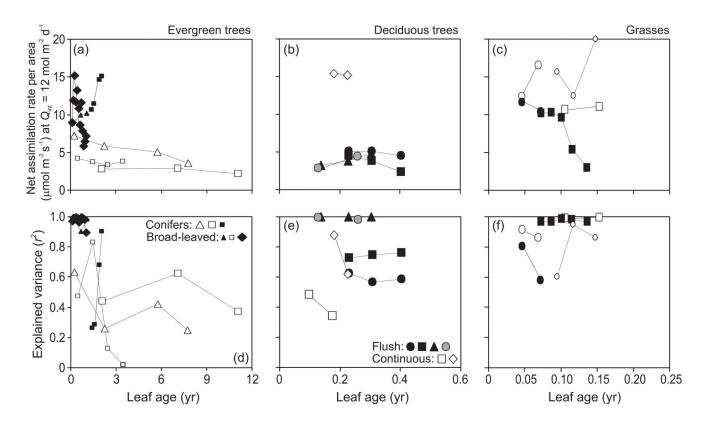


Fig. 8. Age-dependent changes in leaf light-saturated net assimilation rate (photosynthetic capacity) per unit area (A_A) at a constant average daily integrated quantum flux density (Q_{int}) of 12 mol m⁻² d⁻¹ (a-c) and the degree of explained variance (r^2) of Q_{int} vs. A_A dependencies (d-f) in different plant life forms. Sample fits of Q_{int} vs. A_A are shown in Fig. 1c. Symbols used are explained in Fig. 2, and data presentation follows Fig. 3.

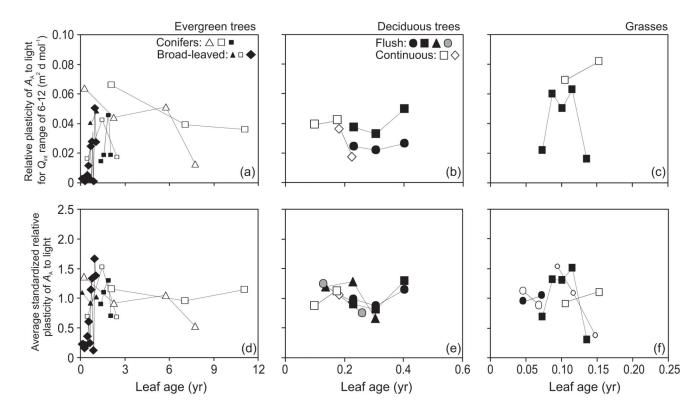


Fig. 9. Leaf age dependent changes in light-dependent plasticity (Eq. 1) of leaf photosynthetic capacity per unit area $(A_{\rm A})$ over the integrated quantum flux density $(Q_{\rm int})$ range of 6-12 mol m⁻² d⁻¹ (a-c) and average standardized plasticity (Eq. 2) calculated over all available light ranges (d-f) in different plant life forms. Symbols as in Fig. 2, presentation of data as in Fig. 3 and plasticity definitions as in Fig. 4. Age-dependent changes in standardized relative plasticity for $Q_{\rm int}$ range of 6-12 mol m⁻² d⁻¹ are depicted in Fig. S2d-f.

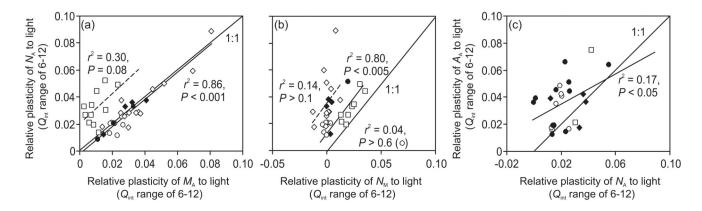


Fig. 10. Relative light-dependent plasticity ($P_{L,l}$) of nitrogen content per area (N_A) in relation to $P_{L,l}$ of leaf dry mass per unit area (M_A ; a) and $P_{L,l}$ of nitrogen content per dry mass (N_M , b) and $P_{L,l}$ of leaf photosynthetic capacity in relation to $P_{L,l}$ of N_A (c). In all cases, $P_{L,l}$ values (m^2 d mol⁻¹) are for the integrated quantum flux density range of 6-12 mol m^{-2} d⁻¹. N_A is the product of M_A and N_M , and thus, the relationships in (a) and (b) demonstrate the controls on N_A plasticity by plasticities of underlying variables. Variation in plasticity estimates is driven both by age-dependent changes and plasticity differences among the canopies included in the analysis (Figs. 4, 6, 7, 9). Data were fitted by linear regressions. In (a), available data for tree species were fitted by a common regression. In (c), all data were fitted by a single regression. One-to-one lines are also demonstrated. Symbols as: ● - evergreen conifers; ○ - evergreen broad-leaved species; - flush-type deciduous broad-leaved species; • - continuous-type deciduous broad-leaved species; □ - forbs.

Tabel 1
List of acronyms used with definitions and corresponding units

Symbol	Definition	Unit
A_{A}	leaf light-saturated net assimilation rate (photosynthetic capacity) per area	μmol m ⁻² s ⁻¹
$A_{ m M}$	leaf light-saturated net assimilation rate per dry mass	μmol g ⁻¹ s ⁻¹
$M_{\rm A}$	leaf dry mass per unit area	g m ⁻²
$N_{\rm A}$	leaf nitrogen content per area	g m ⁻²
$N_{ m M}$	leaf nitrogen content per dry mass	%
$n_{ m L}$	number of leaves in the canopy (Eq. 3)	-
$n_{\rm S}$	leaf number per shoot	-
$P_{ m L}$	relative light-dependent plasticity (Eq. 1)	$\rm m^2~d~mol^{-1}$
$P_{ m L,l}$	$P_{\rm L}$ for the $Q_{\rm int}$ range I (Eq. 1), e.g. $P_{\rm L,6-12}$ refers to the $Q_{\rm int}$ range 6-12 mol m ⁻² d ⁻¹	m^2 d $\mathrm{mol}^{\text{-}1}$
$P_{\mathrm{L,l,j}}$	$P_{ m L,l}$ for the leaf age class j	m^2 d mol^{-1}
$Q_{\rm int}$	daily integrated quantum flux density averaged for specified number of days. In this study, $Q_{\rm int}$ denotes $Q_{\rm int,b50}$ except when noted	mol m ⁻² d ⁻¹
Q _{int,b30}	$Q_{\rm int}$ averaged for 30 days since the start of leaf development or for the actual number of days since the onset of leaf development for leaves younger than 30 days	mol m ⁻² d ⁻¹
$Q_{\rm int,b50}$	as $Q_{\text{int,b30}}$, but averaged for 50 days since the start of leaf development	mol m ⁻² d ⁻¹
$Q_{\rm int,b60}$	as $Q_{\text{int,b30}}$, but averaged for 60 days since the start of leaf development	mol m ⁻² d ⁻¹
$Q_{\rm int,min}$	minimum Q_{int} value observed for the given canopy	mol m ⁻² d ⁻¹
$Q_{\rm int,p50}$	Q_{int} averaged for 50 days preceding leaf sampling or actual number of days when leaves were younger than 50 days	mol m ⁻² d ⁻¹
Q _{int,Rb50}	$Q_{\rm int,b50}$ value corresponding to the year of formation of the most recent leaf flush estimated for older leaf classes in evergreens	mol m ⁻² d ⁻¹
S_{j}	standardize relative trait plasticity for the leaf age class j (Eq. 2)	-
$S_{ m j,l}$	S_j for the Q_{int} range I , e.g. $S_{j,6-12}$ refers to the Q_{int} range 6-12 mol m ⁻² d ⁻¹	-
$ar{\mathcal{S}_{j}}$	average S_j for all light ranges available	-
ā	average leaf age in the canopy	yr
$ar{a}_{ ext{F}}$	\bar{a} at the time of completion of leaf formation in the canopy	yr
$a_{ m C}$	canopy age	yr
φ	phyllochron, time elapsed between the formation of subsequent leaves on the main stem	yr
λ	leaf longevity	yr
$\bar{\lambda}$	species-specific average leaf longevity	yr

Table 2

Summary of the database of studies considering variation in within-canopy leaf plasticity as dependent on leaf age: list of species, number of ages studied, age range considered and availability of traits

Abies amabilis t.en 4 0.25 - 7.76 N _A . A _A (Brooks et al. 1970; et al. 1970) A. veitchii t.en 2 0.29 - 1.29 M _A (Eliásh 1975; et al. 1970) A. seccharum (control) t.wdb 2 0.24 - 0.41 M _A (Eliásh 1975; huzul Eliásh 1975) A. saccharum (control) t.wdb 3 0.23 - 0.40 A _A (Tjoelker et al. 1970) A. saccharum (ozone) t.wdb 3 0.23 - 0.40 A _A (Tjoelker et al. 1970) A. saccharum (ozone) t.wdb 3 0.23 - 0.40 A _A (Tjoelker et al. 1970) Avena fatua g.a 2 0.05 - 0.07 A _A (Barnes et al. 1970) Betula platyphylla (1) t.wdb 4 0.16 - 0.40 M _A (Araki 1973) B. platyphylla (2) t.wdb 5 0.10 - 0.52 M _A (Araki 1973) Carex acutiformis g.p 3 0.10 - 0.52 M _A (Araki 1973) Carpinus betulus t.wdb 2 0.26 - 0.43 M _A (Ar. Na, Na, Mal Eliásh 19	
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G. hirsutum cv. Sicala V-2i (fertilized) f,p $2 0.11 - 0.17$ N_A (Milroy et al.	2001)
Helianthus annuus cv. Panter (1 / control) f,a 5 0.06 - 0.13 N _A (Archontoulis 2011)	et al.
H. annuus cv. Panter (1 / drought) f,a $4 0.08 - 0.13$ N_A (Archontoulis 2011)	et al.
H. annuus cv. Panter (2 / control) f,a $2 0.10 - 0.12$ N_A (Archontoulis 2011)	et al.

Species (replicate/treatment/site) ¹	Life form ²	Number of ages	Age range (yr)	Traits available ³	References
H. annuus cv. Panter (2 / drought)	f,a	4	0.07 - 0.14	$N_{ m A}$	(Archontoulis et al. 2011)
H. annuus cv. Prosol (control/lower density)	f,a	5	0.08 - 0.12	$N_{ m M}$	(Sadras et al. 1993)
H. annuus cv. Prosol (fertilized/lower density)	f,a	5	0.08 - 0.12	$N_{ m M}$	(Sadras et al. 1993)
H. annuus cv. Prosol (control/higher density)	f,a	5	0.08 - 0.12	$N_{ m M}$	(Sadras et al. 1993)
<i>H. annuus</i> cv. Prosol (fertilized/higher density)	f,a	5	0.08 - 0.12	$N_{ m M}$	(Sadras et al. 1993)
Hibiscus cannabinus cv. Everglades 41	f,b	4	0.12 - 0.2	$M_{\rm A}, N_{\rm A}, N_{\rm M}$	(Archontoulis et al. 2011)
Juglans regia	t,wdb	2	0.29 - 0.39	$M_{\rm A}, N_{\rm A}, N_{\rm M}$	(Le Roux et al. 1999)
Mangifera indica ev. Lirfa	t,eb	4	0.13 - 1.54	$M_{\rm A},N_{\rm A},N_{\rm M},A_{\rm A}$	(Urban et al. 2003)
Oryza sativa cv. Norin #18	g,a	5	0.07 - 0.14	$A_{ m A}$	(Takeda and Yajima 1975a, b)
O. sativa cv. Toyonishiki	g,a	2	0.11 - 0.15	$A_{ m A}$	(Sato and Kim 1980a, b, c)
Picea abies	t,en	6	0.21 - 5.21	$M_{ m A}$	(Koppel and Frey 1984)
P. abies	t,en	3	0.34 - 2.34	$M_{\rm A}, N_{\rm A}, N_{ m M}$	(Niinemets 1997a; Niinemets 1997b; Niinemets 1997c)
Picea sitchensis	t,en	2	0.31 - 1.31	$M_{ m A}$	(Lewandowska and Jarvis 1977)
P. sitchensis	t,en	3	0.35 - 2.35	$M_{ m A}$	(Norman and Jarvis 1974)
Pinus contorta var. latifolia	t,en	3	2.09 - 11.1	$N_{ m A},A_{ m A}$	(Schoettle and Smith 1999)
P. densiflora	t,en	5	1.41 - 2.07	$N_{\mathrm{A}}, A_{\mathrm{A}}$	(Han et al. 2004)
P. paustris	t,en	3	0.25 - 0.92	$M_{\rm A},N_{\rm A},N_{\rm M}$	(Niinemets et al. 2002b)
P. sylvestris (infertile site)	t,en	3	0.14 - 2.12	$M_{\mathrm{A}},N_{\mathrm{A}},N_{\mathrm{M}}$	(Niinemets et al. 2002a; Niinemets et al. 2001; Niinemets and Lukjanova 2003)
P. sylvestris (fertile site)	t,en	3	0.32 - 2.34	$M_{\mathrm{A}},N_{\mathrm{A}},N_{\mathrm{M}}$	(Niinemets et al. 2002a; Niinemets et al. 2001; Niinemets and Lukjanova 2003)
P. sylvestris	t,en	2	0.12 - 0.26	$M_{ m A}$	(Hansen et al. 2002)
P. taeda	t,en	3	0.25 - 1.08	$M_{\rm A}, N_{\rm A}, N_{\rm M}$	(Niinemets et al. 2002b)
P. virginiana	t,en	3	0.25 - 0.75	$M_{\rm A}, N_{\rm A}, N_{\rm M}$	(Niinemets et al. 2002b)
Populus tremula	t,wdb	8	0.01 - 0.38	$M_{\rm A},N_{\rm A},N_{\rm M}$	(Niinemets et al. 2004a)
Prunus persica cv. O'Henry	t,wdb	4	0.11 - 0.32	$N_{ m A}$	(DeJong and Doyle 1985)
P. persica cv. Springcrest	t,wdb	4	0.12 - 0.27	$N_{ m A}$	(DeJong and Doyle 1985)
P. persica cv. Alexandra	t,wdb	2	0.10 - 0.18	$M_{\rm A}$, $N_{\rm A}$, $N_{\rm M}$, $A_{\rm A}$	(Walcroft et al. 2002)

Species (replicate/treatment/site) $^{\it I}$	Life form ²	Number of ages	Age range (yr)	Traits available ³	References
Quercus glauca	t,eb	2	0.29 - 0.95	$N_{ m A}$	(Miyazawa et al. 2004)
Q. ilex subsp. ilex	t,eb	4	0.46 - 3.46	$M_{\rm A}, N_{\rm A}, N_{\rm M}, A_{\rm A}$	(Niinemets et al. 2006a)
Q. robur	t,wdb	2	0.13 - 0.27	$M_{ m A}$	(Hansen et al. 2002; Hansen et al. 2003)
Q. robur	t,wdb	2	0.13 - 0.27	$M_{ m A}$	(Hansen et al. 2002; Hansen et al. 2003)
Rhizophora stylosa	t,eb	12	0.12 - 1.04	$A_{ m A}$	(Suwa and Hagihara 2008)
Tilia cordata	t,wdb	8	0.01 - 0.38	$M_{\rm A},N_{\rm A},N_{\rm M}$	(Niinemets et al. 2004a)
Triticum aestivum cv. Apache	g,a	6	0.07 - 0.12	$N_{ m A}$	(Bertheloot et al. 2008)
T. aestivum cv. Isengrain	g,a	6	0.07 - 0.12	NA	(Bertheloot et al. 2008)
T. aestivum cv. Bannock	g,a	2	0.05 - 0.07	$A_{ m A}$	(Barnes et al. 1990; Beyschlag et al. 1990)
T. aestivum cv. Minaret (control/higher density)	g,a	2	0.06 - 0.15	N_{A}	(Dreccer et al. 2000)
T. aestivum cv. Minaret (fertilized/higher density)	g,a	3	0.06 - 0.15	$N_{ m A}$	(Dreccer et al. 2000)
T. aestivum cv. Minaret (control/lower density)	g,a	3	0.06 - 0.15	$N_{ m A}$	(Dreccer et al. 2000)
T. aestivum cv. Minaret (fertilized/lower density)	g,a	3	0.06 - 0.15	$N_{ m A}$	(Dreccer et al. 2000)
Vicia faba var. minuta cv. Tina (higher density)	f,a	2	0.10 - 0.12	$N_{ m A}$	(Del Pozo and Dennett 1999)
V. faba var. minuta cv. Tina (lower density)	f,a	2	0.10 - 0.12	$N_{\rm A}$	(Del Pozo and Dennett 1999)
Xanthium strumarium var. canadense	f,a	3	0.09 - 0.14	$N_{ m A}$	(Hikosaka et al. 1999)
Zea mays cv. hybrid DEA (lower density)	g,a	3	0.07 - 0.10	$N_{ m A}$	(Drouet and Bonhomme 1999)
Z. mays cv. hybrid DEA (higher density)	g,a	3	0.07 - 0.10	$N_{ m A}$	(Drouet and Bonhomme 1999)

¹Species names follow the GRIN taxonomy for plants (USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network - (GRIN), National Germplasm Resources Laboratory, Beltsville, Maryland, http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl? language=en). Replicate studies in time (different year of study) are noted with numeric codes 1-3, and application of treatments (ozonation, fertilization, adjustment of planting density) and presence of replicate sites are also noted. Within-canopy variation in leaf traits was studied in field-grown plants except for the study of Dreccer et al. (2000) where growth-chamber cultivated plants were used. For further details of database construction see Niinemets et al. (2015).

 $^{^2}$ g,a - annual grass; g,b - perennial grass; f,a - annual forb; f,b - biennial forb; f,p - perennial forb; t,wdb - winter-deciduous broad-leaved tree; t,eb - evergreen broad-leaved tree; t,en - evergreen needle-leaved tree (conifer tree)

 $^{^{3}}M_{A}$ - leaf dry mass per unit area; N_{A} - leaf nitrogen content per unit area; N_{M} - leaf nitrogen content per unit dry mass; A_{A} - leaf photosynthetic capacity (light-saturated net assimilation rate) per unit area

Table 3

Pearson correlations (r) of average standardized plasticity $(\bar{S_j})$ for leaf dry mass (M_A) and nitrogen content (N_A) with average leaf age (\bar{a}) in different woody (evergreen conifers and evergreen broad-leaved species, deciduous broad-leaved species) and herbaceous (forbs and grasses) plant life forms

Life form	$\mathcal{S}_{\mathbf{j}}$ for $M_{\mathbf{A}}$ vs. $\mathbf{Log}(ar{m{a}})$			$S_{\mathbf{j}}$ for $N_{\mathbf{A}}$ vs. $\mathbf{Log}(\mathbf{\bar{a}})$				
	r	P	n	Age range (yr)	r	P	n	Age range (yr)
Evergreen conifers	-0.34&	0.065	30	0.12-5.21	-0.40#	0.05	24	0.14-11.1
Evergreen broad-leaved species	-0.36	0.43	7	0.13-2.46	-0.62	0.08	9	0.13-2.46
All evergreens	-0.35	< 0.04	37	0.12-5.21	-0.42	< 0.02	33	0.13-11.1
Deciduous broad-leaved (flush)	0.55	< 0.005	28	0.01-0.43	0.51	< 0.03	19	0.01-0.39
Deciduous broad-leaved (continuous)	0.68	< 0.005	18	0.06-0.35	0.36	0.36	10	0.09-0.44
All deciduous broad-leaved species	0.42	< 0.005	46	0.01-0.43	0.44	< 0.02	29	0.01-0.44
Forbs	-0.14	0.69	10	0.08-0.20	0.34	< 0.03	42	0.06-0.20
Grasses					-0.35*	0.07	29	0.06-0.31

Standardized relative plasticity is defined by Eq. 2. Deciduous broad-leaved species are divided among flush-type species that form a single leaf flush in the beginning of the growing season and continuous-type species that form leaves continuously or in multiple flushes through the growing season

Due to limited data, \bar{S}_1 for M_A vs. $Log(\bar{a})$ statistics for grasses could not be calculated.

[&]amp; r = -0.53, P < 0.005, without an outlying observation of *Picea abies* at $\bar{a} = 5.21$ yr (Fig. 4d)

 $rac{\#}{r} = -0.68$, P < 0.001, without an outlying observation of *Pinus contorta* at $\bar{a} = 2.1$ yr (Fig. 6e)

r = -0.63, P < 0.001 without data for cyperoid grass Carex acutiformis exhibiting an opposite response (Fig. 6h)