**ORIGINAL PAPER**

# Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance

**N. Legner · S. Fleck · C. Leuschner**

## Abstract

***Key message*** **The relative shade tolerance of *T. cordata*, *F. sylvatica*, and *C. betulus* in mature stands is based on different species-specific carbon and nitrogen allocation patterns.**

*Abstract* The leaf morphology and photosynthetic capacity of trees are remarkably plastic in response to intra-canopy light gradients. While most studies examined seedlings, it is not well understood how plasticity differs in mature trees among species with contrasting shade tolerance. We studied light-saturated net photosynthesis ($A_{max}$), maximum carboxylation rate ($V_{cmax}$), electron transport capacity ($J_{max}$) and leaf dark respiration ($R_d$) along natural light gradients in the canopies of 26 adult trees of five broad-leaved tree species in a mixed temperate old-growth forest (*Fraxinus excelsior*, *Acer pseudoplatanus*, *Carpinus betulus*, *Tilia cordata* and *Fagus sylvatica*), representing a sequence from moderately light-demanding to highly shade-tolerant species. We searched for species differences in the dependence of photosynthetic capacity on relative irradiance (RI), specific leaf area (SLA) and nitrogen per leaf area ($N_a$). The three shade-tolerant species (*C. betulus*, *T. cordata*, *F. sylvatica*) differed from the two more light-demanding species by the formation of shade leaves with particularly high SLA but relatively low $N_a$ and consequently lower area-based $A_{max}$, and a generally higher leaf morphological and functional plasticity across the canopy. Sun leaf morphology and physiology were more similar among the two groups. The three shade-tolerant species differed in their shade acclimation strategies which are primarily determined by the species' plasticity in SLA. Under low light, *T. cordata* and *F. sylvatica* increased SLA, mass-based foliar N and leaf size, while *C. betulus* increased solely SLA exhibiting only low intra-crown plasticity in leaf morphology and N allocation patterns. This study with mature trees adds to our understanding of tree species differences in shade acclimation strategies under the natural conditions of a mixed old-growth forest.

**Keywords** *Acer pseudoplatanus* · Adult trees · $A_{max}$ · *Carpinus betulus* · *Fagus sylvatica* · *Fraxinus excelsior* · Relative irradiance · Shade adaptation · Specific leaf area · *Tilia cordata*

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**Abbreviations**

| | |
|---|---|
| $A_{max}$ | Light-saturated net photosynthesis rate under ambient $CO_2$ concentration |
| Ap | *Acer pseudoplatanus* |
| Cb | *Carpinus betulus* |
| $C_i$ | Intercellular $CO_2$ concentration |
| DBH | Diameter at breast height |
| Fe | *Fraxinus excelsior* |
| Fs | *Fagus sylvatica* |
| $J_{max}$ | Maximum electron transport rate |
| $N_a$ | Nitrogen per leaf area |
| $N_m$ | Nitrogen per leaf mass |

| $R_d$ | Leaf dark respiration rate calculated from the $A/C_i$ curve |
|---|---|
| RI | Relative irradiance |
| SLA | Specific leaf area (area per leaf mass) |
| Tc | *Tilia cordata* |
| $V_{cmax}$ | Maximum carboxylation rate |
| VPD | Atmospheric water vapour saturation deficit |
| Suffix 'a' | Scaled to leaf area |
| Suffix 'm' | Scaled to leaf mass |
| Suffix 'N' | Scaled to leaf nitrogen |

## Introduction

Trees represent the plant life form with highest competitive ability for light due to their capability of overgrowing smaller-statured plants. The formation of a tree canopy requires the construction of a high number of structural elements for foliage exposure and it demands for plastic leaf responses to the highly variable irradiance in the canopy (Niinemets 2007). One of the most obvious problems of plants capable of overgrowing their competitors is that the shade cast by their canopy is not only restricting the light access of competitors but also that of the own foliage in the shade crown. While the sun leaves of trees are frequently exposed to full sunlight, shade leaves at the canopy base often have to function with <5 % of incident light (Walter and Torquebiau 1997; Hagemeier 2002; Ellenberg and Leuschner 2010; Niinemets 2010). This dramatic intra-canopy light gradient induces acclimation at the leaf, branch and canopy levels which may vary with species, age, social position in the stand, and environment.

Given the high diversity in leaf forms, leaf life spans, branching patterns and crown dimensions among different tree species (Hallé et al. 1978), it is likely that not a single, but a multitude of competitive strategies for exploring canopy space, capturing light and assimilating $CO_2$ at the stand level do exist. In mixed forests, species with contrasting strategies for capturing aboveground resources often co-exist under similar environmental conditions. In temperate forests, this can be members of the genera *Fagus*, *Tilia*, *Acer*, *Carpinus*, *Fraxinus* and *Quercus* with different phylogenies and functional features. However, we are only beginning to understand how species differences in leaf morphology and physiology, and in the plasticity of leaf functioning are determining the trees' strategies for capturing light and $CO_2$ in species-rich temperate and tropical forests (Rozendaal et al. 2006; Sack et al. 2006; Valladares and Niinemets 2008). Yet, this knowledge is important for a mechanistic understanding of forest dynamics in mixed forests and for the support of foresters in their search for tree species that can increase the yield of mixed stands.

Our current understanding of adult trees' competitive strategies for exploring canopy space and assimilating $CO_2$ is still based on the concept of light-demanding and shade-tolerant species (e.g. Liburnau 1918). However, the concept's limitations have also been recognised since long time (e.g. Fricke-Beutnitz 1904) as it is well known that shade-tolerant species often get more light demanding when growing older while they demand less light when occurring at fertile and warmer sites (Liburnau 1918, Portsmuth and Niinemets 2007). An alternative concept for a functional classification of trees is related to the observed successional status of the species (e.g. Bazzaz 1979). However, key processes in this concept are dispersal, seedling establishment and the competitive success of the offspring which are only partly related to the strategy of light capture and canopy carbon gain in the adult stage.

Tree species differ largely in their capacity to modify leaf morphology, leaf physiology and canopy structure in response to low light (Rozendaal et al. 2006; Sack et al. 2006). Strategies of light-demanding early successional species might result from genetically determined thresholds that constrain the development of highly shade-tolerant foliage by restricting the plasticity of leaf morphology and limiting maximum canopy leaf area, or from a putative maximum leaf area index at the stand level, as it appears, for example, in the pioneer species *Pinus sylvestris* and *Betula pendula* from over-regional LAI surveys (Ellenberg and Leuschner 2010; Niinemets 2010; Bequet et al. 2012). While the development of shade-tolerant foliage is closely linked to efficient light harvesting, which requires the allocation of carbon and nitrogen over larger leaf areas (Niinemets 2010), certain woody species might well face inherent limitations in the maximum leaf size achievable per amount of C and N invested in leaf tissue. Thus, a key factor controlling the adaptability of a tree's foliage is the intra-crown plasticity in leaf area per leaf mass [specific leaf area (SLA)].

While the majority of Central European tree species can adapt or acclimate to only moderate shade intensities (Ellenberg and Leuschner 2010), a few species such as *Fagus sylvatica* (European beech) and *Abies alba* (Silver fir) are able to adjust their foliage to nearly the full spectrum of light availability occurring in forests, i.e. from 100 to approx. 1 % of full sunlight (Tognetti et al. 1997; Valladares et al. 2002; Robakowski et al. 2004). However, most of the broad-leaved trees of this flora do not produce shade leaves at irradiances <10 % of incident radiation (e.g. *Betula*, *Populus*, *Quercus* and *Fraxinus* species) or do not tolerate <5 % relative irradiance (e.g. *Alnus* and *Acer* species) (Hagemeier 2002; Niinemets and Valladares 2006). Why these functional differences between the species do exist, is not sufficiently understood.

Controversial results have been reported concerning the influence of shade tolerance (or successional status) on the light-dependent plasticity in leaf morphology of tree species. For the leaf morphological plasticity of seedlings, either higher plasticity (e.g. Valladares et al. 2002) or lower plasticity (e.g. Oguchi et al. 2005, Strauss-Debenedetti and Bazzaz 1996) in shade-tolerant late-successional species as compared to light-demanding species, or highest plasticity in mid-successional species (Abrams and Mostoller 1995), was found.

For mature trees, it has been postulated that shade-tolerant species should have higher plasticity in traits important for light harvesting (such as SLA and chlorophyll:N ratio), while species adapted to high irradiance should have higher plasticity in leaf traits related to $CO_2$ assimilation (such as $N_a$) (Valladares et al. 2002). However, it is generally assumed that overall plasticity is lower in shade-tolerant species (Valladares and Niinemets 2008) even though this is not fully supported by a number of studies with adult trees (e.g. Rozendaal et al. 2006; Sack et al. 2006). Other factors than shade tolerance may also influence leaf plasticity. For example, Niinemets (2010) postulated that trees with a relatively short leaf life span, which is characteristic for stands with high resource supply (light, water, nutrients), should not be able to produce foliage with distinct shade acclimation.

Progress in our understanding of tree species differences in shade acclimation or adaptation is linked to deeper insights into those factors that promote or limit leaf and canopy modification under low light. Typical morphological changes along the intra-canopy light gradient are increases in leaf size, SLA and chlorophyll content per unit dry mass, and reductions in leaf thickness, the number of palisade mesophyll cell layers, and stomatal density (Lichtenthaler et al. 1981; Givnish 1988; Niinemets 2010). Relevant functional changes with light reduction include a greater fractional investment of N in molecules involved in light harvesting, implying higher light absorptance per dry mass in shade than sun leaves, a reduction in area-specific leaf dark respiration, and reduced investment in light-protecting chemicals associated with the xanthophyll cycle (Niinemets 2007; Valladares and Niinemets 2008). Despite these modifications, tree sun leaves often have been found to reach two- to fourfold higher photosynthetic capacities per leaf area ($A_{max}$) than shade leaves (Hölscher 2004; Le Goff et al. 2004; Urban et al. 2007).

Generally accepted patterns of the functionality of tree crowns are that irradiance is decreasing exponentially with increasing canopy depth (Monsi and Saeki 1953), while irradiance shows a linear positive relation to leaf mass per area (LMA; or a negative hyperbolical one to its inverse, SLA) and also a linear positive relation to nitrogen per leaf area ($N_a$) (Fleck et al. 2004; Niinemets 2007). Area-related

photosynthetic capacity ($A_{max,a}$) increases with $N_a$ either linearly (e.g. Ellsworth and Reich 1993), or as observed in *Fagus sylvatica* and *Quercus petraea* trees (Fleck 2002), asymptotically. Attempts to explain the marked changes in leaf morphology and function along the intra-canopy light gradient prove to be difficult, mostly because SLA (and its inverse, LMA) and $N_a$, as likely key determinants of $A_{max,a}$, are closely related to each other and thus change in parallel with light availability (Meir et al. 2002).

A major methodological problem in the identification of species-specific strategies of canopy light capture and carbon gain in the adult stage is that purely experimental approaches are hardly possible due to the long time span needed for a tree to reach the upper canopy layer in a stand. Observational studies in old-growth mixed stands are a promising alternative tool for comparing the strategies of different tree species under *ceteris paribus* conditions and they have the advantage that crown structure and vertical light gradient in the canopies are in a quasi steady state, thus reflecting the species' strategy of space filling under the influence of decade-long competitive interactions with neighbouring trees.

We started from the hypothesis that the known general pattern of the $A_{max,a}$ dependence on irradiance, SLA and $N_a$ is modified by the environment (e.g. N and water availability) and species-specific traits such as leaf morphological plasticity. We examined the alteration of photosynthetic capacity, SLA, and foliar N content in five co-occurring temperate broad-leaved species in a mixed stand under homogenous microclimatic and edaphic conditions and, thus, were able to search for species differences in the light and nitrogen control of canopy photosynthesis with only minor influence of potentially confounding factors. Moreover, by considering tree species with markedly different shade tolerance of their most basal leaves (highly shade tolerant to moderately light demanding), which is linked to different positions in forest dynamics (late-successional to early-/mid-successional), we attempted to recognise relationships between shade tolerance (or successional status) and the control of canopy photosynthesis by irradiance, SLA and $N_a$.

In conjunction with the study's principal goal, i.e. the search for species-specific strategies of light capture and $CO_2$ assimilation, we tested the following hypotheses: trees capable of producing highly shade-tolerant leaves (1) exhibit a greater leaf morphological plasticity with larger sun-shade leaf differences in lamina size and SLA, and (2) maximise leaf area in the shade crown through a steeper SLA/RI slope as compared to trees lacking characteristic shade leaves; the latter vary less in leaf morphology and tend to maximise N accumulation per leaf area in sun leaves through a steeper $N_m$/SLA slope. We further hypothesised (3) that trees producing highly shade-tolerant

leaves show higher shade leaf photosynthetic capacities per C and N investment ($A_{max}$ or $V_{cmax}$ per leaf mass or per unit leaf N) than trees lacking characteristic shade leaves. Our investigation bases on the comparative analysis of co-variation in leaf morphology, foliar N, photosynthetic capacity ($A_{max}$, $V_{cmax}$, $J_{max}$) and leaf dark respiration ($R_d$) across the canopies of 26 adult trees ($\sim 30$ m tall) from five species of the genera *Fagus*, *Tilia*, *Carpinus*, *Acer* and *Fraxinus* (each five or six representative trees) in an old-growth mixed forest.

## Materials and methods

### Study site

The study was carried out in 2008 and 2009 in Hainich National Park in western Thuringia, Germany. The national park on the southeastern side of the Hainich mountain range is part of the largest unfragmented deciduous forest in Germany with a size of 160 km². The study site consists of a transect of approximately 200-m length and 30-m width in the south-east of the national park near the location Thiemsburg (51°05′00″N, 10°30′27″E) at 350 m a.s.l. The soil type is a Luvisol developed in loess over Triassic Limestone with the topsoil pH ($H_2O$) ranging between 4.5 and 6.8 (C. Langenbruch, personal communications). With up to 14 tree species per hectare, the mixed broad-leaved forest communities of Hainich National Park are among the species-richest Central European forests. While European beech (*Fagus sylvatica* L.) is the most common species in the Hainich forest, species of the genera *Tilia*, *Acer*, *Carpinus*, *Fraxinus*, *Quercus*, *Prunus*, *Ulmus* and others are also present. The more abundant species besides beech are *Tilia cordata* Mill. (small-leaved lime), *Carpinus betulus* L. (European hornbeam), *Fraxinus excelsior* L. (European ash) and *Acer pseudoplatanus* L. (sycamore maple); all five species were selected for study. These species are not only representatives of five different families (Fagaceae, Tiliaceae, Betulaceae, Oleaceae and Aceraceae), but also differ with respect to their natural role in

forest dynamics (*Fagus* is late-successional, *Acer*, *Carpinus* and *Tilia* mid- to late-successional, and *Fraxinus* early- to mid-successional), physiology and crown morphology (Köcher et al. 2009; Ellenberg and Leuschner 2010). The shade tolerance scores given by Niinemets and Valladares (2006) rank the five species in a similar manner as was already postulated by Liburnau (1918), with *F. sylvatica* as the most shade-tolerant species (score 4.56), followed by *T. cordata* (4.18), *C. betulus* (3.97) and *A. pseudoplatanus* (3.73) and finally *F. excelsior* (2.66) as the most light-demanding species.

The growing season 2008 (April 1 to September 30) was characterised by a mean temperature of 14.6 °C and 234 mm of precipitation, that in 2009 by 15.1 °C and 469 mm (data from the weather station Weberstedt/Hainich, Meteomedia, Gais, Switzerland). The average temperature in the year 2008 was 9.5 °C, that in 2009 8.9 °C (long-term mean 7.7 °C). The precipitation total was 500 mm in 2008 and 774 mm in 2009 (long-term mean 590 mm year⁻¹).

### Sampling design and measurements

Each five individuals per species (six in the case of *F. excelsior*) that were similar with respect to demographic status and tree height were selected for study (Table 1). All 26 selected trees were co-dominant mature individuals with their sun crowns reaching the upper canopy layer. The diameter at breast height (DBH at 1.3 m, recorded with D1 dendrometer bands, UMS, Munich, Germany) varied between the five species (species means of 36–50 cm; Table 1), since we attempted to select representative trees of a species in the upper canopy (height $\sim 30$ m), and the species differed in their diameter–height relation. The age of the trees ranged between 80 and 120 years (Gebauer et al. 2008) and tree height between 26 and 31 m (tree height measured with a Vertex inclinometer, Vertex IV, Haglöf Inc., Langsele, Sweden; Table 1). We used a mobile lift (Denka model DL30, Rotlehner Arbeitsbühnen GmbH, Massing-Oberdietfurt, Germany) to access the sun and shade canopies of the trees up to a height of 30 m.

**Table 1** Some characteristics of the five species

| Tree species | No. of tree individuals | Shade tolerance score | Position in succession | DBH (cm) | Tree height (m) |
|---|---|---|---|---|---|
| *Fraxinus excelsior* | 6 | 2.66 | Early/mid | 36.2 ± 2.6 | 27.6 ± 0.9 |
| *Acer pseudoplatanus* | 5 | 3.73 | Mid | 58.3 ± 7.1 | 30.3 ± 0.9 |
| *Carpinus betulus* | 5 | 3.97 | Mid/late | 45.8 ± 4.7 | 26.7 ± 0.7 |
| *Tilia cordata* | 5 | 4.18 | Mid/late | 43.5 ± 5.8 | 27.0 ± 1.1 |
| *Fagus sylvatica* | 5 | 4.56 | Late | 50.0 ± 3.2 | 29.5 ± 0.6 |

Shade tolerance score after Niinemets and Valladares (2006). Position in succession after observations in various forest communities in Central Europe after Ellenberg and Leuschner (2010). DBH is diameter at breast height. DBH and tree height are for the sample trees (mean ± SE)

Due to difficult canopy access and the time-consuming establishment of the $A/Q$ and $A/C_i$ curves, we preferred replicates at the tree level over leaf replicates in the canopy of the same tree which represent pseudoreplicates. The five (six) investigated trees of a species were treated as true replicates because they were 10 up to > 20 m distant to each other (except for two individuals of *F. excelsior* with 5-m distance to each other). All measurements were done on sunny or overcast days (8 a.m. to 5 p.m.) in the summers 2008 (Jun 20–Aug 22) and 2009 (May 29–Aug 29). On every tree, we investigated fully expanded non-detached leaves under in situ conditions in three different canopy layers: uppermost sun canopy layer (canopy openness >0.25), middle canopy (in between upper sun and lower shade canopy), and lower shade canopy (canopy base, canopy openness <0.25). For all leaves, we examined the light and $CO_2$ response of photosynthesis, estimated integrated relative irradiance and determined SLA and $N_a$. Thus, each three leaves per tree were investigated resulting in about 75 leaves examined in total. Since one or two data points had to be excluded, 13–15 leaves per species were considered in the subsequent analysis: 13 in *F. excelsior* and *A. pseudoplatanus*, 14 in *C. betulus* and *F. sylvatica* and 15 in *T. cordata*. The gas exchange measurements were conducted with a portable LI-6400XT IRGA system (LI-COR Inc., Lincoln, Nebraska, USA) equipped with a standard leaf chamber and a LED red/blue light source (type 6400-02B) on intact, attached leaves. We investigated fully expanded healthy and non-senescent leaves of most distal insertion on exposed peripheral twigs in the sun and middle canopy sections, while the leaves of the shade crowns were positioned on branches in the interior of the lower canopy. In the pinnate leaves of *F. excelsior*, a leaflet in the middle part of the leaf was investigated. The $CO_2$ response was investigated through three $A/C_i$ curves per leaf established at leaf temperatures of 19, 25 and 29 °C (±1 °C) under a photosynthetic photon flux density (PPFD) of 2,000 μmol m$^{-2}$ s$^{-1}$. The three measurements may be viewed as replicates at the leaf level, since only one photosynthesis rate was derived by interpolation from the three measurements (for the 25 °C level). Incident PPFD was increased stepwise to full light, and the leaves were allowed to adapt to these conditions for at least 10 min for allowing photosynthesis to stabilise. The IRGA channels were matched before every measuring point. For every $A/C_i$ curve, leaf internal $CO_2$ concentration ($C_i$) was varied in ten steps in the order: 370, 30, 70, 100, 200, 370, 1,200, and 2,000 ppm $CO_2$. The second measurement at 370 ppm was repeated until the measured assimilation rate was approximately the same as in the first measurement at 370 ppm. On the same leaves, a light response curve (at 25 °C and 370 ppm $CO_2$) was established in ten steps: 2,000, 1,200, 500, 200, 100, 50, 20, 0, 0, and 0 μmol m$^{-2}$ s$^{-1}$ PPFD (in

2008 in reverse order). The vapour pressure deficit was held constant at ambient conditions during the measuring campaign on a leaf.

A hemispherical photograph (Nikon Coolpix 8400 with Nikon Fisheye Converter FC–E9) was taken precisely at the position of each leaf for the calculation of canopy openness in the zenith above the leaf, and for deriving an estimate of the integrated relative irradiance received by the leaf during summer. The leaves were brought to the laboratory, scanned, dried (70 °C, 48 h) and weighed for the determination of leaf size (software Winfolia, Regents Instruments, Quebec, Canada) and SLA. The leaf dry mass was analysed for the concentration of N.

We preferred SLA as a leaf morphological parameter over the more widely used LMA, because SLA is more variable in absolute terms in the shade canopy which was in the focus of the study. Both quantities may easily be converted into each other, since LMA equals 1/SLA.

## Data analysis

For characterising the photosynthetic capacity of the leaves, we chose light-saturated net photosynthesis rate ($A_{max}$), maximum carboxylation rate ($V_{cmax}$) and maximum electron transport rate ($J_{max}$) as parameters. In addition, day respiration rate ($R_d$; Farquhar et al. 1980) was calculated from the $A/C_i$ curves. The gas exchange data on the light and $CO_2$ dependence were analysed with non-linear least-square regressions implemented in Mathematica (version 3.0, Wolfram Research, Champaign, IL, USA). For the fitting of the $A/C_i$ curves, the programme RACCIA (Fleck 2002) was used which is based on the equations of Farquhar et al. (1980), Harley and Tenhunen (1991) and Ball et al. (1987). Hence, the rates of $V_{cmax}$ and $J_{max}$ were calculated with the following equations:

$$V_{cmax} = \frac{(A + R_d)\left(C_i + K_c\left(\frac{1+O}{K_c}\right)\right)}{\left(1 - \frac{\Gamma_*}{C_i}\right)C_i} \tag{1}$$

$$J_{max} = \frac{(A + R_d)(4C_i + 8\Gamma_* C_i)}{4(C_i - \Gamma_*)} \tag{2}$$

with $A$ being assimilation rate; $R_d$, leaf day respiration rate; $C_i$, the intercellular $CO_2$ concentration; $K_c$, the Michaelis–Menten constant for carboxylation; O, the oxygen concentration at the site of fixation; and $\Gamma_*$, the $CO_2$ compensation point without consideration of mitochondrial respiration. From the three $A/C_i$ curves established at 19, 25 and 29 °C, $V_{cmax}$ and $J_{max}$ at 25 °C were calculated accordingly.

Light response curves were fitted with a non-rectangular hyperbolic function (Ogren and Evans 1993; Thornley 2002) with the equation

$$A_{max} = -\left(\frac{A\alpha PAR - \xi A^2}{A - \alpha PAR}\right) \tag{3}$$

where $A$ is the assimilation rate at a given photosynthetically active radiation (PAR), $\xi$ a factor which determines the sharpness in the knee of the non-rectangular hyperbolic curve (set to 0.9; Thornley 2002), and $\alpha$ the initial slope of the light response curve (quantum efficiency of photosynthesis).

In cases where more than one leaf was measured for gas exchange because the first leaf broke away due to strong wind, we used the N content and leaf size data of the leaf that was analysed for the 25 °C $A/C_i$ curve. Most parameters were expressed not only in relation to leaf area, but also to leaf mass. All photosynthetic parameters were analysed for a dependence on time (i.e. day of the year); none of the parameters showed such a dependency (data not shown).

Relative irradiance was derived from hemispherical photographs based on the method of Niinemets and Fleck (2002) and using the model of Roderick (1999) to estimate the proportion of diffuse radiation in global radiation. Global radiation measurements were performed on a tower in about 1 km distance to the study area. The coefficients used in the Roderick model were $X_0 = 0.21$, $X_1 = 0.71$, $Y_0 = 0.95$ and $Y_1 = 0.24$. The fraction of diffuse radiation in global radiation ($f_{dif}$) was 72.8 and 64.6 % in the period of leaf unfolding of the years 2008 and 2009, respectively, and the fraction of direct radiation ($f_{dir}$), thus, 27.2 and 35.4 %. Leaf unfolding was assumed to have occurred during the period May 15–June 30 in both years. The diffuse ($I_{dif}$) and direct site factor ($I_{dir}$) of the leaves were calculated for the relevant period with the programme GLA (version 2.0, SFU, Burnaby, Canada), using a cloudiness index of 0.46 and a clear sky transmission coefficient of 0.6. Relative irradiance (RI) was then calculated as

$$RI = I_{dif} \times f_{dif} + I_{dir} \times f_{dir}. \tag{4}$$

While most calculations were conducted with all leaves in the continuum from sun to middle and shade crown, a few analyses were conducted with the subpopulations of 'sun leaves' and 'shade leaves' to examine the size of the vertical gradient in the canopy. In this approach, we treated all leaves growing at a canopy openness <0.25 as shade leaves and >0.25 as sun leaves. The choice of a canopy openness threshold of 0.25 is supported by the curvature of the SLA/relative irradiance plots which show marked slope alteration in this range (see Fig. 2a). The gas exchange data were tested for a dependence on the time of measurement (June, July or August). As no significant seasonal effect existed, we pooled the data.

Since not all data set met the assumptions for ANOVA, we applied a permutation test to test for significant differences between means among the five species and between sun and shade leaves (significance level:

$P < 0.05$) using the software environment R, version 2.0.8 (R Development Core Team 2008). We used the package 'perm' (Fay and Shaw 2010) with permKS and permTS. Differences with a significance level of $0.05 < P < 0.1$ were treated in the text as 'marginally significant'. Differences between the slopes of linear regression equations (ds) were tested for significance with the software environment SAS 9.2 (SAS Institute, Cary, NC, USA) using the linear model function. Regression analyses were conducted with the software SigmaPlot (version 11.0, Systat Software, Inc., San Jose, California, USA).

While most relationships between leaf properties were treated as being linear, non-linear models were calculated wherever the nature of the relationship is known from earlier investigation. Thus, the relation between height in the canopy and relative irradiance was treated as being exponential (Monsi and Saeki 1953), that one between RI and SLA as being hyperbolic (e.g. Niinemets et al. 2004), and that one between SLA and $N_a$ or parameters of area-related photosynthetic capacity as hyperbolic as well. The relationship between photosynthetic capacity and $N_a$ was assumed to follow a sigmoid curve according to measurements done by Fleck (2002).

When regressing area-related $A_{max}$, $V_{cmax}$, $J_{max}$ and $R_d$ and mass-related $A_{max}$ on RI, SLA or $N_a$ for a relative comparison of influential factors, we tested both linear and non-linear models but presented only those with highest explanatory power. We did not include DBH or tree height as an influential variable in any of the analyses because light availability has been found to exert a much stronger effect on intra-canopy gradients of leaf morphology than tree height and related DBH (Sack et al. 2006; but see Thomas 2010).

Phenotypic plasticity was calculated as an index (ranging from 0 to 1) for each variable and species as the difference between the maximum and minimum value in the canopy divided by the maximum value (Valladares et al. 2002).

## Results

### Intra-canopy gradients of leaf morphology and foliar N in the five species

Relative irradiance (RI) as calculated from hemispherical photographs inside the crown showed the expected exponential decrease from canopy top to bottom with a similar shape of the curve in all five species (Fig. 1a, b). However, early- to mid-successional *F. excelsior* differed from the other four species in the lack of shade leaves at relative irradiances $\lesssim 0.1$.

In all five species, SLA showed a linear increase with decreasing relative height in the canopy ($R^2$ 0.47–0.79;

**Fig. 1** Change of relative irradiance (RI) with absolute height in the canopy (**a**), and of RI, SLA and $N_a$ with relative height in the canopy (**b**, **c** and **d**) for the five species. The equations and their parameters are given in Table 3 (columns 1–3)
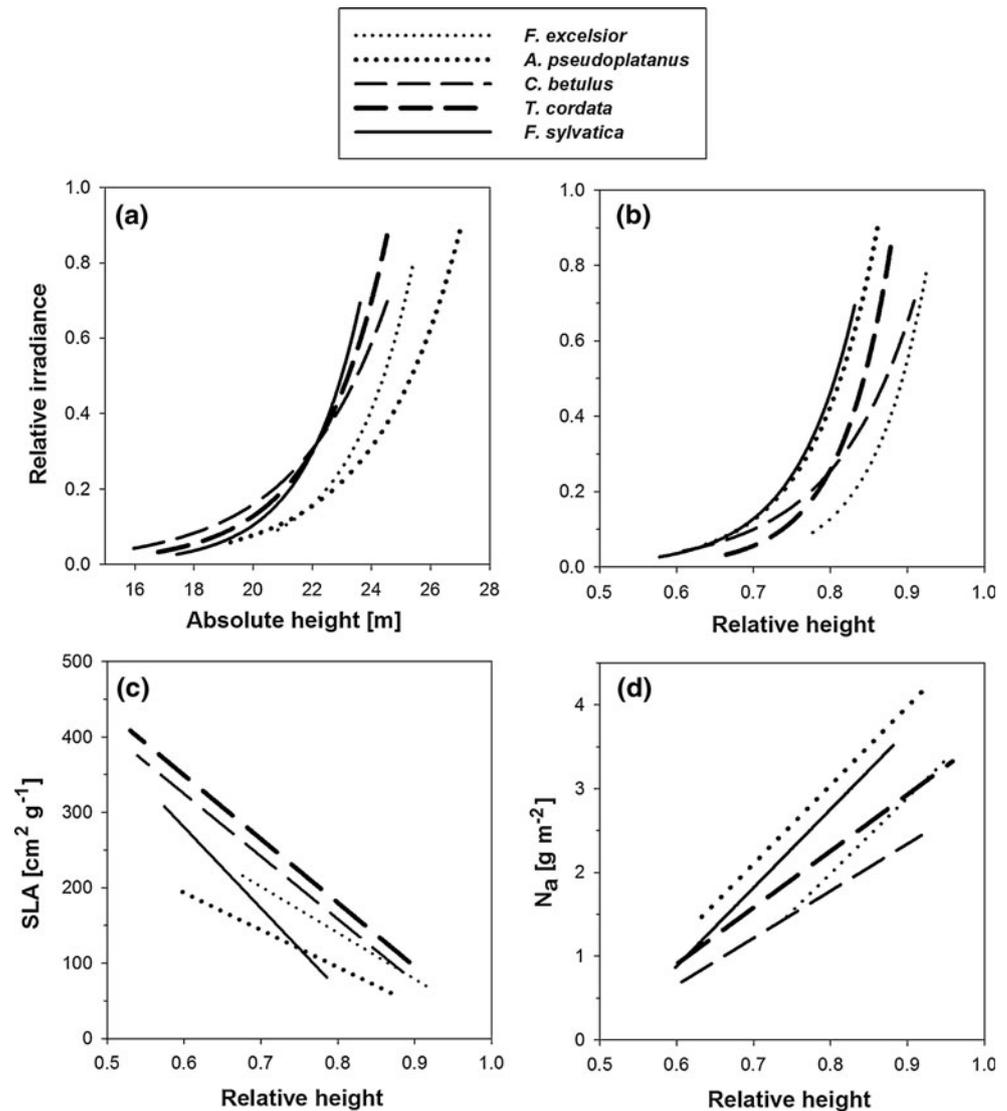


Fig. 1c) but the slope was steeper in *T. cordata*, *C. betulus* and *F. sylvatica* than in *F. excelsior* and *A. pseudoplatanus* (differences significant to Fagus) (Table 3: column 2). SLA decreased hyperbolically with increasing RI ($R^2$ 0.71–0.84; Fig. 2a), with a steeper slope in the more shade-tolerant species *F. sylvatica*, *C. betulus* and *T. cordata* than in the more light-demanding species *F. excelsior* and *A. pseudoplatanus*. This difference corresponded to narrower observed SLA ranges in *F. excelsior* and *A. pseudoplatanus* (60–221 cm$^2$ g$^{-1}$) than in the other three species (83–409 cm$^2$ g$^{-1}$; Figs. 1c and 2a). Only the latter three species produced shade leaves with specific leaf areas well exceeding 250 cm$^2$ g$^{-1}$ (Fig. 2a).

As an average over the whole canopy, the leaves of *A. pseudoplatanus* had higher $N_a$ values (mean 2.6, range 1.5–4.2 g N m$^{-2}$) than those of *T. cordata* and *F. sylvatica* (means 2.1 and 2.1, range 0.9–3.3 and 0.9–3.5 g N m$^{-2}$, respectively; Figs. 1d and 2b; Table 2; difference only partly

significant at $P < 0.05$). *C. betulus* differed from the four other species by relatively low $N_a$ values with a more restricted range of variation (mean 1.5, range 0.7–2.4 g m$^{-2}$; Fig. 2b).

$N_a$ increased linearly with both RI ($R^2$ 0.63–0.89) and (though less tightly) with relative height in the canopy ($R^2$ 0.44–0.77; Figs. 2b and 1d). The slope was steepest in *A. pseudoplatanus* ($N_a$ vs. RI and relH) and in *F. sylvatica* ($N_a$ vs. relative height) and showed no obvious relation to the shade tolerance or successional status of the species. Mass-related nitrogen content ($N_m$) was generally higher in *T. cordata* leaves ($P < 0.05$), but remarkably similar among the four other species (sun leaf $N_m$ means between 22.2 and 25.9 mg N g$^{-1}$, shade leaf means: 24.7–28.4 mg g$^{-1}$, Table 2). In general, $N_m$ remained relatively constant throughout the crown; in two species, however, we found a slight increase in $N_m$ with a RI reduction (significant relation in *F. sylvatica*, a marginally significant one in *T. cordata*, Fig. 2c).
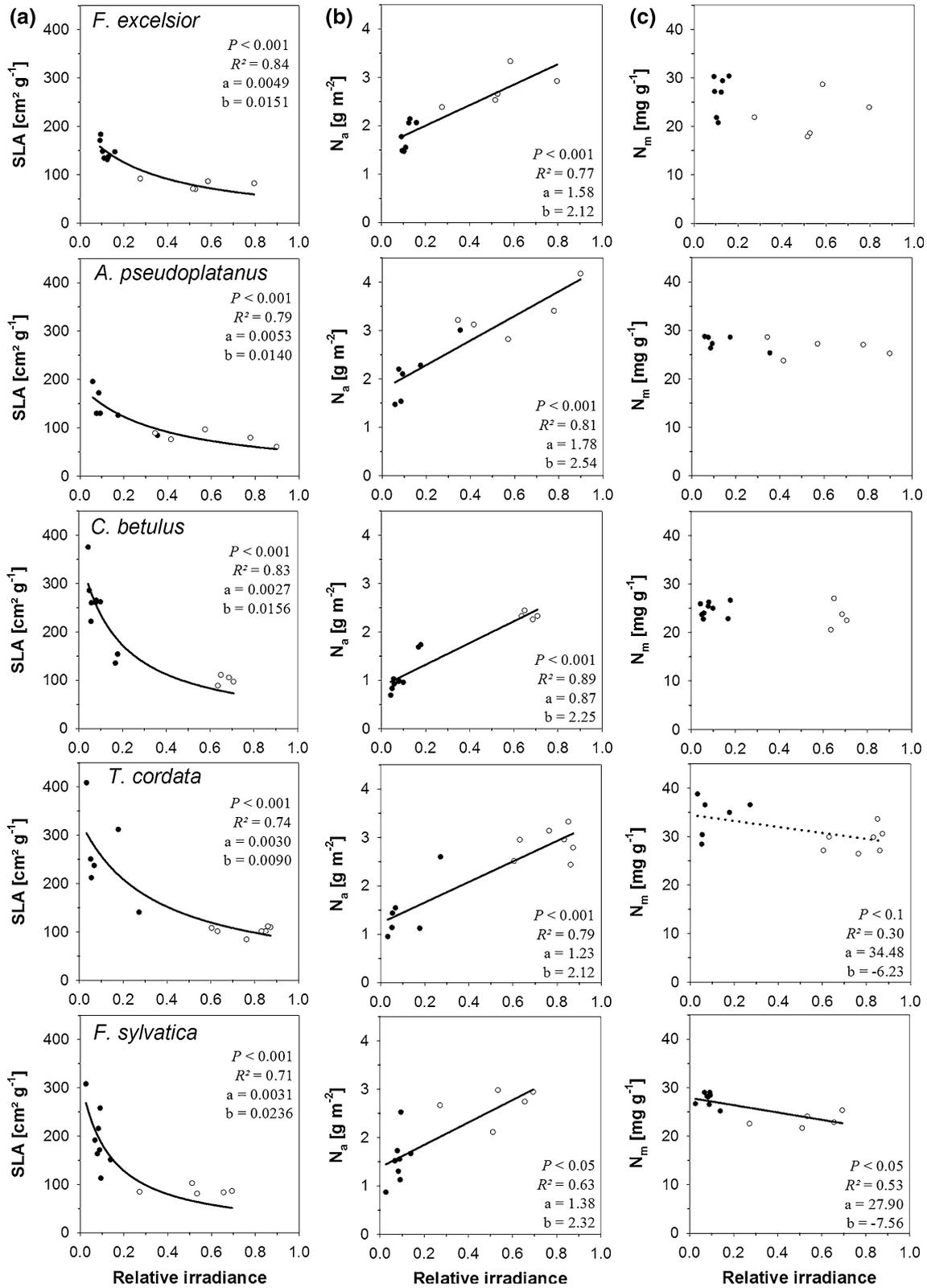
**Fig. 2** Relationship between relative irradiance (RI) and SLA (**a**), $N_a$ (**b**) or $N_m$ (**c**) for the sun (*open circles*) and shade (*filled circles*) leaves of the five tree species. Given are $P$, $R^2$ and the coefficients $a$ and $b$ of the regression equations (SLA: $y = 1/(ax + b)$; $N_a$ and $N_m$: $y = bx + a$). *Solid lines* mark significant relationships ($P < 0.05$), *dotted lines* those with $0.05 < P < 0.1$

**Table 2** Leaf morphological and physiological parameters for 'sun leaves' and 'shade leaves' of the five species (mean ± SE of $n$ leaves)

| Species | $n$ | Leaf size (cm$^2$) | SLA (cm$^2$ g$^{-1}$) | $N_a$ (g m$^{-2}$) | $N_m$ (mg g$^{-1}$) | $V_{cmax,a}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$) | $J_{max,a}$ (μmol e- m$^{-2}$ s$^{-1}$) | $A_{max,a}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$) | $R_{d,a}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Fe sun | 5 | 246.5 ± 38.3[a,A] | 80.1 ± 4.3[a,A] | 2.8 ± 0.2[a,A] | 22.2 ± 2.0[a,A] | 73.6 ± 9.5[a,A] | 155.9 ± 14.0[a,A] | 14.3 ± 2.3[a,A] | 1.9 ± 0.3[a,A] |
| Fe shade | 8 | 201.9 ± 33.3[a,Γ] | 159.2 ± 10.9[b,Γ] | 1.8 ± 0.1[b,Γ,Θ] | 27.8 ± 1.7[b,Γ,Θ] | 56.3 ± 5.5[b,Γ] | 99.7 ± 7.7[b,Γ,Θ] | 14.3 ± 1.4[a,Γ] | 1.3 ± 0.1[b,Γ] |
| PI | | 0.949 | 0.683 | 0.559 | 0.498 | 0.620 | 0.609 | 0.580 | 0.769 |
| Ap sun | 6 | 73.7 ± 8.8[a,B] | 79.2 ± 5.1[a,A] | 3.3 ± 0.2[a,B] | 25.9 ± 0.8[a,A] | 75.5 ± 6.4[a,A] | 175.3 ± 12.7[a,A] | 13.3 ± 1.9[a,A] | 1.8 ± 0.4[a,A] |
| Ap shade | 7 | 88.2 ± 7.4[a,Θ] | 147.2 ± 15.5[b,Γ] | 2.0 ± 0.2[b,Γ] | 28.4 ± 1.0[a,Γ] | 61.5 ± 6.3[a,Γ] | 141.7 ± 18.9[a,Γ] | 13.3 ± 1.3[a,Γ,Θ] | 1.6 ± 0.3[a,Γ] |
| PI | | 0.601 | 0.691 | 0.648 | 0.303 | 0.614 | 0.662 | 0.662 | 0.801 |
| Cb sun | 5 | 26.5 ± 3.4[a,C] | 100.7 ± 3.8[a,B] | 2.3 ± 0.0[a,C] | 23.5 ± 1.1[a,A] | 56.7 ± 3.7[a,A] | 115.8 ± 7.0[a,A] | 12.7 ± 1.1[a,A] | 1.1 ± 0.1[a,A] |
| Cb shade | 9 | 29.3 ± 1.8[a,Δ] | 246.6 ± 23.8[b,Δ,Θ] | 1.1 ± 0.1[b,Δ] | 24.7 ± 0.5[a,Θ] | 33.2 ± 5.6[b,Δ] | 63.6 ± 9.3[b,Δ] | 7.1 ± 1.7[b,Δ] | 0.9 ± 0.1[a,Γ] |
| PI | | 0.538 | 0.764 | 0.717 | 0.239 | 0.775 | 0.773 | 0.845 | 0.897 |
| Tc sun | 7 | 27.4 ± 3.5[a,C] | 102.5 ± 3.4[a,B] | 2.9 ± 0.1[a,AB] | 29.3 ± 1.0[a,B] | 64.6 ± 2.6[a,A] | 151.0 ± 14.4[a,A] | 15.8 ± 2.6[a,A] | 1.5 ± 0.2[a,A] |
| Tc shade | 8 | 47.6 ± 3.8[b,Θ] | 293.9 ± 35.2[b,Δ] | 1.3 ± 0.2[b,Δ,Θ] | 35.3 ± 1.5[b,Δ] | 44.7 ± 7.6[b,Γ,Δ] | 85.2 ± 14.9[b,Δ,Θ] | 8.5 ± 1.9[b,Θ] | 1.3 ± 0.2[a,Γ] |
| PI | | 0.686 | 0.793 | 0.724 | 0.358 | 0.732 | 0.794 | 0.916 | 0.646 |
| Fs sun | 6 | 19.0 ± 2.3[a,C] | 86.9 ± 3.2[a,A] | 2.8 ± 0.2[a,AB] | 24.3 ± 1.1[a,A] | 64.5 ± 4.4[a,A] | 119.8 ± 23.2[a,A] | 7.5 ± 2.3[a,A] | 2.2 ± 0.6[a,A] |
| Fs shade | 8 | 29.9 ± 1.8[b,Δ] | 196.4 ± 22.1[b,Γ,Θ] | 1.5 ± 0.2[b,Γ,Θ] | 27.6 ± 0.5[b,Γ] | 50.8 ± 4.8[a,Γ] | 93.6 ± 12.9[a,Γ,Δ] | 9.5 ± 1.1[a,Δ] | 1.2 ± 0.2[a,Γ] |
| PI | | 0.682 | 0.737 | 0.754 | 0.262 | 0.636 | 0.882 | 0.864 | 0.813 |

Sun leaves refer to leaves exposed to a canopy openness >25 %, shade leaves to an openness <25 %. PI is the plasticity index after Valladares et al. (2002). Different lower case letters indicate significant differences between the sun and shade leaves of a species ($P < 0.05$, permutation test), differences between the sun leaves of the five species are marked with different uppercase letters, those between the shade leaves with different Greek letters ($P < 0.05$, permutation test)
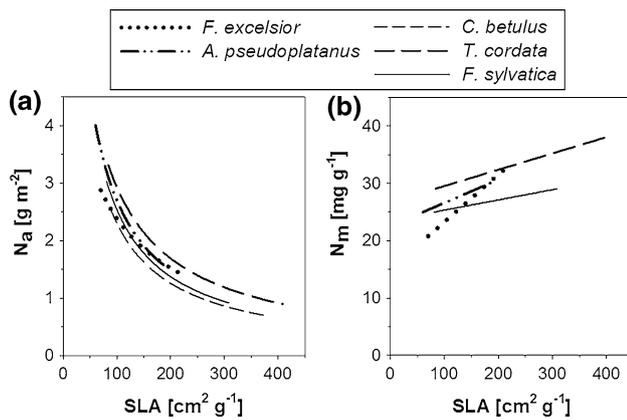
**Fig. 3** Relationships between SLA and $N_a$ (**a**), and SLA and $N_m$ (**b**) in the canopies of the five tree species. Only significant (or marginally significant) relations are printed. The equations and their parameters are given in Table 3 (columns 4 and 5)

Examining the inter-relationships between $N_a$ or $N_m$ and SLA as likely determinants of photosynthetic capacity showed that $N_m$ was positively related to SLA in four species (significant in *F. excelsior*, *A. pseudoplatanus* and *T. cordata*, marginally significant in *F. sylvatica*, no relation in *C. betulus*; Fig. 3b; Table 3: column 5). Across all species, $N_m$ showed a closer relation to SLA than to relative irradiance (Table 3: column 5; Fig. 2c). Nevertheless, the $N_m$/SLA relation was less tight than the negative hyperbolical relation between $N_a$ and SLA (Fig. 3a; Table 3: column 4) which was the closest relationship between any two of the leaf traits investigated ($P < 0.001$ in all species, $R^2$ values of 0.69 to 0.96). The slope parameter $b$ ranged between 0.0025 and 0.0036 in the five species and showed no clear relation to the shade tolerance of the species.

While all species had higher $N_a$ and smaller SLA values in their sun than shade leaves (Table 2), the sun leaves of four species had also a smaller size than the shade leaves (difference significant in *T. cordata* and *F. sylvatica*, non-significant trend in *A. pseudoplatanus* and *C. betulus*). Early- to mid-successional *F. excelsior* differed from the other species by a non-significant tendency for larger, and not smaller, sun than shade leaves (Table 2). SLA increased and $N_a$ decreased with increasing leaf size in *T. cordata* and *F. sylvatica*, but no such relationship was found in the other three species (Table 4). Only in these two moderate-to-high shade-tolerant species, leaf size showed a significant negative dependence on relative irradiance. $N_m$ decreased with increasing leaf size in *F. excelsior*, but increased in *F. sylvatica* (Table 4). The plasticity index after Valladares et al. (2002) for SLA and $N_a$ was higher for the shade-tolerant than for the more light-demanding species (Table 2).

Intra-canopy gradients of photosynthetic capacity and leaf dark respiration and their relation with RI, $N_a$ and SLA

The species differences in the area-related photosynthetic capacity of sun leaves (i.e. leaves exposed to >25 % canopy openness) were only moderate and not significant in our species sample (Table 2). However, shade-tolerant *F. sylvatica* tended to have a lower $A_{\max,a}$ mean and *F. sylvatica* and *C. betulus* a lower $J_{\max,a}$ mean in the sun canopy than the other species (differences not significant at $P < 0.05$). The species differences were more pronounced in the shade leaves (<25 % canopy openness) where the species with higher light demand (*F. excelsior* and *A. pseudoplatanus*) had higher $A_{\max,a}$ and $J_{\max,a}$ means (differences to the other species mostly significant) and tended to have higher $V_{\mathrm{cmax},a}$ means than the more shade-tolerant *C. betulus*, *T. cordata* and *F. sylvatica* (Table 2). The plasticity index of Valladares et al. (2002) for $J_{\max}$ and $A_{\max}$ was higher in the shade-tolerant than in the more light-demanding species (Table 2).

While the $V_{\mathrm{cmax},a}/J_{\max,a}$ ratio tended to be higher in shade than in sun leaves of *F. excelsior* (not significant) and *T. cordata* (significant at $P < 0.05$), no difference was found in *A. pseudoplatanus*, *C. betulus* and *F. sylvatica* (Table 5). The $V_{\mathrm{cmax},a}/J_{\max,a}$ ratio showed neither a relation to the shade tolerance of the species nor to the morphological sun/shade leaf difference.

Table 6 presents the results of regression analyses on the dependence of photosynthetic capacity (area-related $V_{\mathrm{cmax}}$, $J_{\max}$ and $A_{\max}$ and mass-related $A_{\max}$) and (area-related) leaf dark respiration on RI, SLA and $N_a$. The expected positive relation between relative irradiance and photosynthetic capacity was found to be significant for $V_{\mathrm{cmax}}$, $J_{\max}$ and $A_{\max}$ only in two species (*C. betulus* and *T. cordata*); in *A. pseudoplatanus* and *F. excelsior*, only the RI–$J_{\max}$ relation was significant but not the relation to $A_{\max}$ and $V_{\mathrm{cmax}}$ (marginally significant for *F. excelsior*). In *F. sylvatica*, only $V_{\mathrm{cmax},a}$ increased significantly (and $J_{\max,a}$ marginally significantly) with increasing irradiance (Table 6).

$V_{\mathrm{cmax},a}$ and $J_{\max,a}$ showed significant negative relations to SLA and positive relations to $N_a$ in all species (except for $V_{\mathrm{cmax}}$ in *F. excelsior*: non-significant relation to SLA; and $J_{\max}$ in *F. sylvatica*: marginally significant relations to SLA and $N_a$). The SLA and $N_a$ influence was generally weaker on $A_{\max,a}$ than on $V_{\mathrm{cmax},a}$ and $J_{\max,a}$: this relation was significant only in *C. betulus* and *T. cordata*, but non-significant in the other three species (*F. excelsior*, *A. pseudoplatanus* and *F. sylvatica*). In the latter species, SLA, $N_a$ and also RI significantly influenced mass-related $A_{\max}$ instead of area-related $A_{\max}$.

We compared the five species with respect to three measures of photosynthetic performance across the intra-

**Table 3** Results of regression analyses for the dependence of RI, SLA and $N_a$ on relative height (columns 1–3), for the relation between SLA and $N_a$ or $N_m$ (columns 4 and 5), and for the dependence of $V_{cmax,m}$ on SLA, $A_{max,N}$ on RI, and $A_{max,m}$ on $N_m$ (columns 6–8) in the five species

| Regression equation | (1) RI vs. relH $y = 10^{-5}a\ exp(bx)$ | (2) SLA vs. relH $y = a + bx$ | (3) $N_a$ vs. relH $y = a + bx$ | (4) $N_a$ vs. SLA $y = 1/(a + bx)$ | (5) $N_m$ vs. SLA $y = a + bx$ | (6) $V_{cmax,m}$ vs. SLA $y = a + bx$ | (7) $A_{max,N}$ vs. RI $y = a + bx$ | (8) $A_{max,m}$ vs. $N_m$ $y = a + bx$ |
|---|---|---|---|---|---|---|---|---|
| *F. excelsior* | | | | | | | | |
| P | **0.0023** | **0.0016** | **0.0129** | **0.0004** | **0.0045** | **0.014** | **0.0212** | **0.0035** |
| $R^2$ | **0.62** | **0.61** | **0.44** | **0.69** | **0.54** | **0.44** | **0.46** | **0.59** |
| a | **0.1148** | **630.10** | **−5.25** | **0.1812** | **14.98** | **298.61** | **122.33** | **−86.12** |
| b | **14.525** | **−612.53** | **9.04** | **0.0025** | **0.0829** | **3.71** | **−81.59** | **10.51** |
| ds | na | **Fs*** | ns | na | **Tc*.Fs** | ns | **Fs*** | ns |
| *A. pseudoplatanus* | | | | | | | | |
| P | **0.0143** | **0.0008** | **0.0032** | **<0.0001** | **0.0069** | **0.0163** | **0.0106** | **0.0493** |
| R | **0.50** | **0.66** | **0.56** | **0.96** | **0.50** | **0.42** | **0.53** | **0.31** |
| a | **2.423** | **489.48** | **−4.47** | **0.0657** | **22.5** | **410.5** | **98.69** | **−140.7** |
| b | **12.216** | **−493.69** | **9.39** | **0.0030** | **0.041** | **2.84** | **−56.05** | **10.62** |
| ds | n.a. | **Fs*** | (Cb) | na | ns | ns | ns | ns |
| *C. betulus* | | | | | | | | |
| P | **<0.0001** | **0.0007** | **<0.0001** | **<0.0001** | 0.1569 | 0.6998 | 0.8122 | 0.1697 |
| $R^2$ | **0.80** | **0.63** | **0.77** | **0.96** | 0.16 | 0.01 | 0.01 | 0.16 |
| a | **14.371** | **823.69** | **−2.72** | **0.0723** | 22.7 | 629.17 | 80.18 | −121.88 |
| b | **9.3478** | **−831.68** | **5.62** | **0.0036** | 0.008 | 0.21 | 7.18 | 10.76 |
| ds | na | **Fs** | (Ap, Fs) | na | na | na | na | na |
| *T. cordata* | | | | | | | | |
| P | **0.0177** | **0.0002** | **0.0036** | **<0.0001** | **0.0013** | **0.0033** | 0.8895 | 0.1794 |
| $R^2$ | **0.58** | **0.79** | **0.63** | **0.95** | **0.56** | **0.5** | 0.002 | 0.14 |
| a | **0.1347** | **857.06** | **−3.14** | **0.0907** | **26.7** | **598.87** | 84.10 | −35.95 |
| b | **15.2004** | **−846.41** | **6.75** | **0.0025** | **0.029** | **1.56** | −4.65 | 7.1 |
| ds | na | **Fs** | ns | na | **Fe*** | ns | na | na |
| *F. sylvatica* | | | | | | | | |
| P | **<0.0001** | **0.0092** | **0.0016** | **<0.0001** | *0.0723* | **0.0046** | *0.0722* | 0.1121 |
| $R^2$ | **0.90** | **0.47** | **0.61** | **0.92** | *0.24* | **0.53** | *0.26* | 0.2 |
| a | **1.5989** | **924.82** | **−4.73** | **0.0604** | *23.5* | **446.67** | *92.14* | −209.05 |
| b | **12.84** | **−1074.17** | **9.35** | **0.0033** | *0.018* | **2.22** | *−79.08* | 12.96 |
| ds | na | **all species** | (Cb) | na | *Fe** | ns | *Fe*** | na |

Given are $P$, $R^2$, the equation parameters *a* and *b*, and the significance of species differences in the slope (ds) of the relationships. Significant relations in bold, marginally significant ones (0.05 < P < 0.1) in italics

*relH* relative height, *RI* relative irradiance, *ns* not significant, *na* not applicable due to non-linear relationship

canopy light gradient, the $V_{cmax,m}$–SLA relation, the $A_{max,m}$–$N_m$ relation and the dependence of $A_{max,N}$ on RI (Fig. 4). (1) Maximum carboxylation rate per leaf mass ($V_{cmax,m}$) increased with SLA (relationship significant in all species except for *C. betulus*), but showed steeper slopes (2.8 and 3.7 μmol m$^{-2}$ s$^{-1}$) in the more light-demanding species *A. pseudoplatanus* and *F. excelsior* than in the more shade-tolerant *T. cordata* and *F. sylvatica* (1.6 and 2.2 μmol m$^{-2}$ s$^{-1}$; Table 3: column 6; Fig. 4a); however, this difference was not significant. (2) $A_{max,m}$ increased with foliar N concentration ($N_m$) in *F. excelsior* and *A.*

*pseudoplatanus*, while $N_m$ had no influence on the $A_{max,m}$ variation in the canopies of the more shade-tolerant species (Table 3: column 8; Fig. 4c). (3) Instantaneous photosynthetic N use efficiency (PNUE or $A_{max,N}$) decreased with increasing irradiance in *F. excelsior*, *A. pseudoplatanus* and (marginally significant) *F. sylvatica* (Fig. 4b; Table 3: column 7) but revealed no relation in the other species.

Leaf day respiration showed no clear picture of interrelationships with relative irradiance, SLA and $N_a$ among the five species (Table 6). The expected positive relation

**Table 4** Results of linear regression analyses between leaf size and relative irradiance (RI), SLA, $N_a$ or $N_m$

|  | RI | SLA (cm² g⁻¹) | $N_a$ (g m⁻²) | $N_m$ (mg g⁻¹) |
|---|---|---|---|---|
| *F. excelsior* | | | | |
| P | 0.8736 | *0.0765* | 0.8546 | **0.0050** |
| $R^2$ | 0.003 | *0.26* | 0.003 | **0.53** |
| Direction | + | − | + | − |
| *A. pseudoplatanus* | | | | |
| P | 0.1437 | 0.7128 | 0.3672 | 0.2459 |
| $R^2$ | 0.22 | 0.01 | 0.07 | 0.12 |
| Direction | − | + | − | − |
| *C. betulus* | | | | |
| P | 0.6357 | 0.9168 | 0.8895 | 0.1301 |
| $R^2$ | 0.02 | 0.0009 | 0.002 | 0.18 |
| Direction | − | + | − | + |
| *T. cordata* | | | | |
| P | **0.0019** | **0.0021** | **0.0002** | 0.1652 |
| $R^2$ | **0.60** | **0.53** | **0.68** | 0.14 |
| Direction | − | + | − | + |
| *F. sylvatica* | | | | |
| P | **0.0045** | **0.0333** | **0.0234** | **0.0343** |
| $R^2$ | **0.53** | **0.32** | **0.36** | **0.32** |
| Direction | − | + | − | + |

Given are P, $R^2$ and the direction of the relationship (+ positive, − negative). Significant relations in bold, marginally significant ones (0.05 < P < 0.1) in italics

**Table 5** $V_{cmax,a}/J_{max,a}$ ratio (in mol mol⁻¹) of sun and shade leaves of the five tree species and the total leaf sample

|  | Sun leaves | Shade leaves | All leaves |
|---|---|---|---|
| *F. excelsior* | 1.86 ± 0.10[a,A] | 2.26 ± 0.14[a,Γ] | 2.11 ± 0.11[A,B] |
| *A. pseudoplatanus* | 1.82 ± 0.31[a,A] | 1.78 ± 0.08[a,Γ] | 1.80 ± 0.14[A] |
| *C. betulus* | 1.98 ± 0.17[a,A] | 2.06 ± 0.15[a,Γ] | 2.03 ± 0.11[A,B] |
| *T. cordata* | 1.80 ± 0.17[a,A] | 2.12 ± 0.09[b,Γ] | 1.97 ± 0.10[A,B] |
| *F. sylvatica* | 2.47 ± 0.41[a,A] | 2.52 ± 0.43[a,Γ] | 2.50 ± 0.30[B] |

Different lower case Latin letters indicate significant differences between sun and shade leaves for a species; different upper case Latin letters give significant species differences for sun leaves and 'all leaves'; different Greek letters indicate species differences for shade leaves

between $N_a$ and area-related leaf day respiration was found in only one species (*F. sylvatica*) together with a marginally significant relation in *C. betulus*. However, leaf day respiration expressed on a leaf mass basis ($R_{d,m}$) scaled positively with N per leaf mass ($N_m$) in three species (*F. excelsior*, *T. cordata*, *F. sylvatica*) and with SLA in two species (*F. excelsior*, *T. cordata*, data not shown) indicating a higher mass-specific dark respiration of the shade

**Table 6** Results of regression analyses between relative irradiance (RI), SLA or $N_a$, and five gas exchange parameters

|  | $V_{cmax,a}$ | $J_{max,a}$ | $A_{max,a}$ | $A_{max,m}$ | $R_{d,a}$ |
|---|---|---|---|---|---|
| **Relative irradiance (RI)** | | | | | |
| *F. excelsior* | | | | | |
| P | *0.0853** | **0.0026*** | 0.7273* | **0.0479** | **0.0448*** |
| $R^2$ | *0.27* | **0.61** | 0.01 | **0.37** | **0.34** |
| Direction | + | + | + | − | + |
| *A. pseudoplatanus* | | | | | |
| P | **0.0223*** | **0.0001*** | 0.2972* | **0.0052** | 0.1843* |
| $R^2$ | **0.46** | **0.82** | 0.12 | **0.60** | 0.19 |
| Direction | + | + | + | − | + |
| *C. betulus* | | | | | |
| P | **<0.0001*** | **<0.0001*** | **0.0001*** | 0.9832 | *0.0841** |
| $R^2$ | **0.78** | **0.80** | **0.78** | 4.7 10⁻⁵ | *0.25* |
| Direction | + | + | + | − | + |
| *T. cordata* | | | | | |
| P | **0.0047*** | **0.0019*** | **0.0178*** | 0.5791 | 0.1512* |
| $R^2$ | **0.53** | **0.60** | **0.45** | 0.03 | 0.18 |
| Direction | + | + | + | − | + |
| *F. sylvatica* | | | | | |
| P | **0.0154*** | *0.0699** | 0.6340* | **0.0279** | 0.5211* |
| $R^2$ | **0.46** | *0.29* | 0.02 | **0.37** | 0.04 |
| Direction | + | + | + | − | + |
| **SLA** | | | | | |
| *F. excelsior* | | | | | |
| P | 0.1813 | **0.0056[a]** | 0.6897 | **0.0183[a]** | 0.2482 |
| $R^2$ | 0.15 | **0.52** | 0.02 | **0.44** | 0.12 |
| Direction | − | − | − | + | − |
| *A. pseudoplatanus* | | | | | |
| P | **0.0335** | **0.0085[a]** | 0.3087 | **0.0128[a]** | 0.3243 |
| $R^2$ | **0.34** | **0.48** | 0.09 | **0.44** | 0.09 |
| Direction | − | − | − | + | − |
| *C. betulus* | | | | | |
| P | **0.0002[a]** | **<0.0001[a]** | **0.001[a]** | 0.5516 | **0.0137** |
| $R^2$ | **0.74** | **0.80** | **0.66** | 0.03 | **0.41** |
| Direction | − | − | − | − | − |
| *T. cordata* | | | | | |
| P | **0.0002[a]** | **0.0002[a]** | **0.0071[a]** | 0.3661 | 0.5453 |
| $R^2$ | **0.70** | **0.69** | **0.47** | 0.07 | 0.03 |
| Direction | − | − | − | + | − |
| *F. sylvatica* | | | | | |
| P | **0.0021[a]** | *0.0686* | 0.8543 | **0.0221[a]** | *0.0644[a]* |
| $R^2$ | **0.61** | *0.27* | 0.003 | **0.36** | *0.27* |
| Direction | − | − | + | + | − |
| **$N_a$** | | | | | |
| *F. excelsior* | | | | | |
| P | **0.0312** | **0.0033** | 0.4514 | 0.2714 | 0.1374 |
| $R^2$ | **0.50** | **0.68** | 0.16 | 0.25 | 0.19 |
| Direction | + | + | + | + | + |

**Table 6** continued

|  | $V_{cmax,a}$ | $J_{max,a}$ | $A_{max,a}$ | $A_{max,m}$ | $R_{d,a}$ |
|---|---|---|---|---|---|
| *A. pseudoplatanus* |  |  |  |  |  |
| *P* | **0.0084** | **0.0067** | 0.4304 | **0.0049** | 0.4046 |
| $R^2$ | **0.62** | **0.63** | 0.16 | **0.66** | 0.06 |
| Direction | + | + | + | + | + |
| *C. betulus* |  |  |  |  |  |
| *P* | **<0.0001** | **0.0001** | **<0.0001** | 0.2976 | *0.0973* |
| $R^2$ | **0.87** | **0.81** | **0.87** | 0.22 | *0.21* |
| Direction | + | + | + | + | + |
| *T. cordata* |  |  |  |  |  |
| *P* | **<0.0001** | **0.0005** | **0.0207** | 0.6587 | 0.1407 |
| $R^2$ | **0.79** | **0.72** | **0.51** | 0.07 | 0.16 |
| Direction | + | + | + | + | + |
| *F. sylvatica* |  |  |  |  |  |
| *P* | **0.0019** | *0.0709* | 0.4908 | **0.0014** | **0.0051** |
| $R^2$ | **0.71** | *0.41* | 0.12 | **0.70** | **0.53** |
| Direction | + | + | − | + | + |

Given are $P$, $R^2$ and the direction of the relationship (+ positive, − negative). Equations for RI: linear equations except for cases marked by * (non-linear regression equation: $y = a\,(1 - \exp^{(-bx)})$); equation for SLA: $y = 1/(a + bx)$; equation for $N_a$: $y = ax^b/(x^b + c)$; linear equation for $R_d$ (SLA and $N_a$). Significant relations in bold, marginally significant ones ($0.05 < P < 0.1$) in italics

[a] Marks parameters with a significant linear regression to LMA

leaves in these species, which contrasts with the generally lower area-specific respiration of the shade leaves (see Table 2).

# Discussion

## Species differences in low-light adaptation: leaf morphology and foliar N

Our comparison of the five tree species revealed larger species differences in leaf morphology and physiology in the shade crown than in the sun crown. Leaves exposed to a canopy openness >0.25 ('sun leaves') showed only moderate species differences in SLA, nitrogen per area and mass, photosynthetic capacity per area ($V_{cmax}$, $J_{max}$ and $A_{max}$) and leaf day respiration. For the majority of parameters, these differences were not significant and no clear relation to the species' shade tolerance or successional status was visible. A marked exception was only sun leaf size with a high variation among the species. Thus, the five species revealed more or less convergent patterns in the morphology and functioning of the sun leaves despite large differences in phylogeny, crown morphology, shade tolerance, and physiology (Köcher et al. 2009). One factor that certainly has favoured convergence in the sun foliage
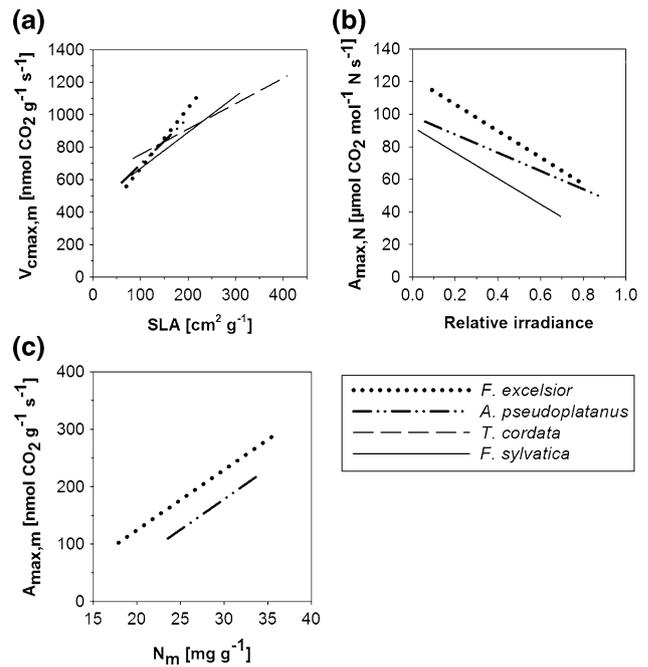


**Fig. 4** Dependence of $V_{cmax,m}$ on SLA (**a**), of $A_{max,N}$ (photosynthetic N use efficiency) on relative irradiance (**b**), and of $A_{max,m}$ on $N_m$ in the canopies of the five species. Only significant (or marginally significant) relationships are printed. The equations and their parameters are given in Table 3 (columns 6–8)

is the uniform microclimatic and edaphic conditions under which the species existed in the Hainich mixed forest.

The situation is different in the shade canopy (here defined as canopy openness <0.25, RI typically <0.2) where the species can be arranged into two groups according to SLA, $N_a$ and the photosynthetic performance of the shade leaves, the first group with higher light demand (*F. excelsior*, *A. pseudoplatanus*), the second with moderate-to-high shade tolerance (*C. betulus*, *T. cordata*, *F. sylvatica*). A key variable determining the formation of adapted shade leaves is certainly SLA that reached in the first group maximum values of only ~200 cm² g⁻¹, which is considerably less than the maximum value found in the more shade-tolerant second group. Since *Fraxinus* and *Acer* have on average much larger shade (and also sun) leaves than *C. betulus*, *T. cordata* and *F. sylvatica* (*A. pseudoplatanus*: two- to fourfold; *F. excelsior*: four- to 12-fold larger; Table 2), it is likely that specific leaf area cannot further be increased in the large *Fraxinus* and *Acer* leaves. The limits of maximising leaf area by increasing SLA and reducing leaf thickness are set by the mechanical requirements of the foliage and its exposure to additional stresses such as drought, herbivory and frost (Valladares and Niinemets 2008). This explanation is supported by our observation that the lowest irradiance levels measured in proximity to *F. excelsior* and *A. pseudoplatanus* shade leaves were higher (RI not below 0.05 or 0.03) than those

recorded near typical shade leaves of the more shade-tolerant three species (RI minima of 0.02 or 0.01, see Fig. 2a). This suggests that ash and maple are indeed less shade tolerant than the other three species as is also indicated by the scores of relative shade tolerance of Niinemets and Valladares ([2006]).

The $N_a$ minima in the shade leaves were much lower in *C. betulus*, *T. cordata* and *F. sylvatica* than in *F. excelsior* and *A. pseudoplatanus*. While the large leaves of ash and maple may be costly in terms of supporting structures, those of *T. cordata* are characterised by particularly high N investments per leaf mass (significantly higher $N_m$ values than in the other species). Lime shade leaves also had the highest SLA values of all species examined; thus, with respect to morphology, these leaves must be considered as the most distinct shade leaves in our species sample. The large SLA variation in the leaves of *T. cordata* was associated with a high plasticity in leaf size; lime shade leaves were nearly twice as large as sun leaves.

Due to their small size and relatively low N concentration, *F. sylvatica* and *C. betulus* shade leaves are apparently the least costly leaves in terms of N investment and supporting structures. However, the two species differ in that *F. sylvatica* manages to reach high $N_a$ values in its sun leaves due to a much higher plasticity in leaf size, while *C. betulus*, which apparently is neither flexible in leaf size nor in N investment per leaf mass, produces the sun leaves with lowest $N_a$ values of all species investigated. Thus, most leaves in the canopy of *C. betulus* have to be considered as shade leaves in terms of SLA and $N_a$, while the leaves of *T. cordata* and *F. sylvatica* cover a wide range of morphological types with sun leaves of these shade-tolerant species being similar to the sun leaves of light-demanding species.

As expected, $N_a$ and SLA were closely related to each other in a negative hyperbolical way in all species (Fig. 3a). This relation resulted not only from the intra-canopy gradient in lamina thickness (or SLA), but it is also partly caused by changes in mass-specific N content ($N_m$) from the sun to the shade crown. We found significant $N_m$ increases with increasing SLA in *F. excelsior*, *A. pseudo-platanus* and *T. cordata*, and a tendency for an $N_m$ increase in *F. sylvatica*. Correspondingly, $N_m$ decreased significantly with increasing relative irradiance in *F. sylvatica* (and marginally significant also in *T. cordata*), evidencing higher N concentrations in shade than sun leaves in three of the investigated species. This matches with results obtained by Niinemets ([1997]) in four broad-leaved tree species who assumed that $N_m$ should be more responsive to decreasing irradiance in shade-tolerant than in light-demanding species because an increase of light-harvesting compounds per leaf mass and, thus, in the specific absorptivity of shade leaves is one strategy to relieve light limitation imposed on

photosynthesis. This assumption is supported by our data where the $N_m$ increase with decreasing RI was only found in the highly (or moderately) shade-tolerant species *F. sylvatica* and *T. cordata*. In these species, shade leaves represent a high-priority sink for nitrogen.

Our results on leaf morphological plasticity within the canopies contradict the assumption that shade-tolerant tree species should generally be less plastic than light-demanding species (Valladares and Niinemets [2008]). A larger within-canopy plasticity of the shade-tolerant species is not only visible in the higher plasticity indices for SLA, $N_a$, $J_{max}$ and $A_{max}$ in this group, but also in the steeper SLA increase with increasing depth in the canopy in these species as compared to more light-demanding *F. excelsior* and *A. pseudoplatanus*.

It has been pointed out that leaf plasticity in trees depends on ontogeneity, tree size and also radiation regime (Rozendaal et al. [2006]; Valladares and Niinemets [2008]). Empirical studies on within-canopy gradients in leaf morphology, foliar N and photosynthetic capacity in mature closed forests are rare (e.g. Kull and Niinemets [1998]; Niinemets et al. [1999]; Meir et al. [2002]). One should expect that the shade exposure of the most basal leaves is higher, and the trait differences between sun and shade leaves are larger, in closed stands with tall trees than in younger stands or isolated trees. That tree size affects the intra-crown plasticity in leaf morphology is suggested from a comparison of the Hainich data with other published results that found lower plasticity in the foliage. For example, the 27-m tall *T. cordata* trees of our study had up to four times higher SLA in shade than in sun leaves, while Niinemets et al. ([2004]) reported an only twofold difference in 4–17 m tall lime trees. Differences in the radiation regime may also be influential; this could explain the apparent discrepancy between our results and those of Sack et al. ([2006]) from six temperate broad-leaved tree species. These authors found no relation between intra-canopy leaf plasticity and the species' light requirements for regeneration, which are typically related to the shade tolerance of the adult trees. Sack et al. ([2006]) studied 13–18 m high solitary trees in an arboretum and not ~30 m tall trees in a closed forest as we did.

## Species differences in low-light adaptation: photosynthetic capacity and leaf respiration

As for leaf morphology, we found larger species differences in the physiology of shade than sun leaves. The lowest shade leaf photosynthetic and respiratory activity among the five species was found in *C. betulus*, the species with the least variable leaves in terms of mass-specific N investment ($N_m$); this species' sun leaf activity was also relatively small. The limited plasticity in $N_m$, leaf size and

also in mass-specific $A_{max}$ and the lacking relation of $A_{max,m}$ to relative irradiance, SLA or $N_a$ in *C. betulus* indicates that this species seems to be relatively inflexible with respect to the allocation of photosynthesis-relevant compounds to leaf tissues. This species achieves acclimation or adaptation to variable light availabilities in the crown mainly through alteration in SLA, and not by modifying $N_m$ and/or leaf size, which is an important strategy in the other four species.

*Tilia cordata* has in common with *C. betulus* that SLA variation plays an important role for achieving photosynthetic acclimation across the light availability gradient as is indicated by the lacking influence of RI, SLA and $N_a$ on mass-based $A_{max}$ in both species, whereas RI, SLA and $N_a$ had a strong influence on area-based $A_{max}$. However, *T. cordata* was much more flexible with respect to $N_m$ and also leaf size than *C. betulus* resulting in higher photosynthetic capacities per area (and also per mass) in the sun leaves of lime than hornbeam.

*Fraxinus excelsior*, *A. pseudoplatanus* and *F. sylvatica* have in common that area-based $A_{max}$ was, contrary to our expectation, not dependent on relative irradiance, SLA and $N_a$ while mass-based $A_{max}$ showed a significant relation to these determinants of photosynthetic capacity. This indicates that the increase in leaf thickness (i.e. the SLA decrease) from the shade to the sun leaves was not large enough in these three species to compensate the decline in mass-specific photosynthetic capacity from the shade to the sun leaves, as it is indicated by the negative $A_{max,m}$–RI relation in Table 6. Three factors may be discussed as potential causes of a reduced mass-based photosynthetic capacity in the trees' sun leaves, (i) a smaller investment of N in leaf structures involved in light harvesting in sun leaves as compared to shade leaves (which explains the reduced photosynthetic N use efficiency of sun leaves, Fig. 4b), (ii) a reduced $N_m$ concentration (as found in *F. sylvatica*), and (iii) higher leaf internal diffusion limitation (Niinemets 2007) and reduced light availability in the interior of the thicker sun leaves.

From the $V_{cmax,m}$ increase with SLA in Fig. 4a, it appears that the light-demanding species *F. excelsior* and *A. pseudoplatanus* are more efficiently increasing the $CO_2$ net flux into the leaf with a growth in leaf area when the leaves become thinner (higher SLA) as compared to the more shade-tolerant species *F. sylvatica* and *T. cordata*, even though the latter reached higher $V_{cmax,m}$ values in absolute terms in their shade leaves (see Fig. 4a).

Our data further suggest a shift in the major abiotic determinants of photosynthetic capacity, RI, SLA and $N_a$, from the more light-demanding to the shade-tolerant species. In the most light-demanding species *F. excelsior*, all three factors correlated closely with $J_{max,a}$ (and to a lesser degree with $V_{cmax,a}$), while in the most shade-tolerant species *F. sylvatica*, the closest correlations were found with $V_{cmax,a}$ (and only weakly with $J_{max,a}$). In the other three species with intermediate shade tolerance, RI, SLA and $N_a$ were correlated with both $V_{cmax,a}$ and $J_{max,a}$. This might imply that electron transport capacity was optimised with respect to light availability in the more light-demanding species, while it was the carboxylation capacity that was optimised in the more shade-adapted species.

The inspection of three parameters characterising photosynthetic capacity per unit of invested carbon or nitrogen gave, however, no indication that the shade leaves of the more shade-tolerant species (*C. betulus*, *T. cordata* and *F. sylvatica*) were generally more efficient in terms of resource use than those of the more light-demanding species: neither mass-specific $V_{cmax}$ or $A_{max}$, nor photosynthetic N use efficiency ($A_{max,N}$) were generally higher in extreme shade leaves of the group of the more shade-tolerant species (see Figs. 4a–c). This was only the case in *T. cordata* with significantly higher $V_{cmax,m}$ and $J_{max,m}$ rates in the shade leaves than in the other species (Legner et al. 2013). For achieving a more general picture of physiological shade leaf adaptation in temperate trees, a larger number of tree species have to be investigated.

### Determinants of photosynthetic capacity

$N_a$ was in all species (except for $J_{max}$ in *A. pseudoplatanus*) a better predictor of $V_{cmax}$ and $J_{max}$ ($R^2$ values of 0.41–0.87) than relative irradiance or SLA ($R^2$ 0.27–0.82). In contrast, a relation of $A_{max,a}$ to these three parameters existed only in *C. betulus* and *T. cordata*, but was nonsignificant in the other three species. This result is evidence for significant species differences in the strategies of leaf morphological and functional adaptation to light availability gradients. Tree species which only increase SLA (such as *C. betulus*) may be contrasted with species that are capable of increasing SLA and also foliar N concentration (such as *T. cordata* and *F. sylvatica*). The increase in $N_m$ under low light is probably a main driver of the observed linear increase in mass-related maximum carboxylation rate ($V_{cmax,m}$) with SLA in four of the five species of our study, but it was lacking in *C. betulus* with its inflexible N allocation strategy. A corresponding increase in $V_{cmax,m}$ towards the shade leaves was found by Meir et al. (2002) in the canopies of tall temperate broad-leaved and tropical trees.

It is unexpected that only two species (*C. betulus*, *T. cordata*) showed a dependence of $A_{max,a}$ on $N_a$, but three did not. Other studies on intra-canopy light gradients found a linear correlation between $N_a$ and $A_{max}$ (e.g. Ellsworth and Reich 1993). However, there are some reasons to assume that photosynthetic capacity is not always closely related to the absolute amounts of N per leaf area along

intra-canopy light gradients. Maximum photosynthesis in deep shade may depend more on SLA, leaf angles and the mode of N partitioning between chemicals used either for light harvesting or for carboxylation (Meir et al. 2002) than on absolute amounts of N per leaf area; these effects could weaken the $N_a$ influence on $A_{max}$. In the species without a clear $N_a$ influence on $A_{max}$ in our study (*F. excelsior*, *A. pseudoplatanus* and *F. sylvatica*), we instead found a significant positive relationship between mass-specific $A_{max}$ and $N_a$. This may be viewed as an indication that N partitioning must have played a prominent role for determining the intra-canopy variation in photosynthetic capacity, while a larger effect of $N_a$ than of $N_m$ on photosynthetic capacity may point at a key role of SLA. This implies that leaf structural modifications are not the only means by which temperate trees are adjusting to low light, but that alteration in N partitioning can also be influential which is not in line with the assumption of Niinemets (2007) that trees modify mostly SLA.

The generally higher $N_m$ values of the shade leaves as compared to the sun leaves in the species examined may in part explain why mass-specific photosynthetic capacity ($V_{cmax,m}$; $J_{max,m}$; $A_{max,m}$) in four of the five species (exception: *C. betulus*) was higher in shade than in sun leaves in the Hainich forest (see plot of $V_{cmax,m}$ vs. SLA in Fig. 4a) which contrasts with the area-specific rates. Our findings contradict earlier studies in broad-leaved trees that found mass-specific $J_{max}$ and $A_{max}$ to be relatively invariant along the intra-canopy light gradient (e.g. Ellsworth and Reich 1993; Niinemets, Kull and Tenhunen 2004), but which are consistent with the higher mass-specific photosynthetic capacities in shade than in sun leaves reported by Niinemets et al. (1998). However, these studies investigated smaller trees and thus shorter intra-canopy light gradients than we did. A factor that might also be influential is the rather high fertility of the Hainich soils; we argue that high soil N availability could promote relatively high shade leaf N concentrations as were found in our study.

Respiratory losses per dry mass were generally higher in shade than in sun leaves corresponding to the elevated N concentrations. As a consequence, area-related leaf day respiration rate did not significantly decrease with increasing shade tolerance of the species, not supporting the prediction of the carbon gain hypothesis formulated by Givnish (1988).

Other causes of intra-canopy leaf modification

An examination of canopy architecture and the general ecology of the five species (Frech et al. 2003; Ellenberg and Leuschner 2010) indicate that traits related to canopy architecture may also play an important role in determining the degree of shade adaptation in these five species. In the Hainich forest, Frech (2006) found a longer vertical crown extension in the species with moderate-to-high shade tolerance (*C. betulus*, *T. cordata*, *F. sylvatica*) than in light-demanding *F. excelsior* which concentrated the foliage in the upper third of canopy space. Possible explanations for this discrepancy are that leaves and branches in the lower part of the ash canopy are missing because (i) the C and N resources needed to build the foliage are preferentially invested in the sun crown for maximising carbon gain in full light, or (ii) the species is inherently limited in its potential to produce shade leaves with higher SLA and better low-light adaptation (Sack et al. 2006; Niinemets 2007; Valladares and Niinemets 2008).

The inter-relationship between leaf morphology, N partitioning and photosynthetic capacity is further complicated by the fact that the intra-canopy light gradient is typically associated with vertical gradients in atmospheric water vapour saturation deficit (VPD), leaf water status, wind speed and leaf temperature, factors that can modify the light influence on leaf morphology and function. Elevated VPD may not only limit photosynthesis through a reduction of stomatal conductance (Gries 2004), but it can also influence leaf expansion growth and may determine final leaf size in trees (Fender et al. 2011). Further abiotic stressors such as excess radiation, restricted water supply and high leaf temperatures can reduce the assimilation rate of sun leaves and may have negative effects on leaf expansion (Niinemets et al. 1999; Chaves et al. 2002; Niinemets 2007). Experiments under field conditions would be needed to prove the importance of these factors for leaf morphology and function.

## Conclusions

In our species sample, the three shade-tolerant species *C. betulus*, *T. cordata* and *F. sylvatica* differed from the two more light-demanding species in a number of leaf- and canopy-level traits relevant for low-light acclimation. Among the most important differences are a greater vertical canopy extension and, in support of hypothesis (1), a higher intra-canopy SLA variation in the shade-tolerant species, which is reflected in a relatively steep SLA/RI slope and in the formation of shade leaves with high SLA (exceeding values of $\sim 250$ cm$^2$ g$^{-1}$), supporting hypothesis (2). Shade leaf $N_a$ was relatively low in the shade-tolerant species due to a high SLA, even though two species increased mass-specific N content ($N_m$) with decreasing light. In shade-tolerant temperate broad-leaved trees, a plurality of low-light adaptation strategies seems to exist. In fact, the three shade-tolerant species *C. betulus*, *T. cordata* and *F. sylvatica* differed markedly with respect to the relative importance of light-induced modifications in either SLA or N allocation patterns to leaf tissues. Species-

specific restrictions in the capability of achieving high SLA values are certainly a key trait. We found no convincing support for our hypothesis (3) that the shade leaves of the more shade-tolerant species should be more efficient in terms of C and N use in photosynthesis than those of the light-demanding species.

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