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Role of foliar nitrogen in light harvesting and shade tolerance of four temperate deciduous woody species

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Summary

1. Distribution of leaf chlorophyll (Chl), nitrogen and non-structural carbohydrates (NSC, ethanol-soluble sugars and starch) in leaves along a natural light gradient was studied in saplings of four deciduous woody species: *Acer platanoides*, *Padus avium*, *Populus tremula* and *Quercus robur* (from most shade tolerant to intolerant).
2. Leaf dry mass per area (LMA) increased linearly with increasing relative irradiance (RI) in all species and therefore it may be considered as a species-specific estimate of long-term light conditions.
3. Decreasing irradiance enhanced chlorophyll synthesis, and consistently resulted in highest Chl per leaf dry mass in low-light environments. Chl content per leaf area was correlated negatively with RI in *A. platanoides* and was independent of RI in three other species.
4. Leaf nitrogen content per leaf area (N_a) increased linearly with LMA in all species, and with RI in all except *A. platanoides*.
5. The slopes and intercepts of N_a vs LMA relationships were negatively correlated. Moreover, it appeared that the slope decreased and the intercept increased with increasing species shade tolerance. Thus, average nitrogen content per mass (N_m) increases and maximum concentrations of leaf nitrogen shift towards more open habitats with decreasing shade tolerance. Distribution of foliar nitrogen in this way is likely to contribute to greater photosynthetic potentials at higher irradiances in shade-intolerant species. Inversely, more tolerant species have greater concentrations of foliar nitrogen at low irradiance — an investment pattern, which provides resources to enhance the capacity of light harvesting in light-limited conditions.
6. NSC per leaf dry mass was positively related to relative irradiance and the slope of this relationship was highest for shade-intolerant species, which have intrinsically higher photosynthetic capacities. Nevertheless, the conclusions were robust and were not significantly biased by using the parameters expressed either on a total or on a NSC-free dry mass.
7. The analysis was further extended by including a number of other species from several independent studies. Examination of both sets of data resulted in identical conclusions. Based on this evidence it may be concluded that the variability in leaf nitrogen concentration in relation to irradiance plays a central role in species shade tolerance.

Key-words: *Acer platanoides*, chlorophyll, irradiance, leaf morphology, *Padus avium*, *Populus tremula*, *Quercus robur*

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Introduction

Changes in structural and physiological properties of foliage, which enhance the efficiency of light interception, gain in importance as the light available to fuel photosynthesis becomes progressively deficient with increasing depth in the canopy. Therefore, plant performance is optimized if the foliage morphology

and physiology are adjusted towards long-term light conditions across the canopy (Tooming 1967; Björkman 1981; Givnish 1988; Gutschick & Wiegel 1988; Baldocchi & Harley 1995). Acclimation to growth irradiance plays an even more significant part in forest understorey, where light is the most universally limiting resource for all leaves (Bazzaz & Wayne 1994; Pearcy & Sims 1994).

Nitrogen availability and species-specific distribution patterns of foliar nitrogen in relation to irradiance may crucially alter the plant's capacity to tolerate low irradiances, because nitrogen scales with thylakoid proteins and leaf chlorophylls (Evans 1989, 1993a). While the content of leaf nitrogen per leaf area (N_a) is positively related to relative irradiance (Walters & Field 1987; Lemaire *et al.* 1991; Kull & Niinemets 1993; Niinemets 1995, 1997), the relationships between nitrogen content per leaf dry mass (N_m) and relative irradiance differ qualitatively among temperate deciduous species: N_m increases with increasing irradiance or decreasing canopy depth in *Betula pendula* (Kull & Niinemets 1993), is constant in *Acer rubrum* (Harley & Baldocchi 1995), *Prunus persica* (DeJong & Doyle 1985) and *Quercus alba* (Harley & Baldocchi 1995), but decreases in *Acer saccharum* (Ellsworth & Reich 1992), *Corylus avellana* (Kull & Niinemets 1993), *Fagus sylvatica* (Niinemets 1995) and *Lonicera xylosteum* (Kull & Niinemets 1993). Diverse responses of N_a and N_m with respect to irradiance can be explained by introducing an important structural parameter, leaf dry mass per area (LMA), which enables interconversion between the mass and area-based estimates ($N_a = N_m \times \text{LMA}$), and which is positively related to growth irradiance (Gulmon & Chu 1981; Kull & Niinemets 1993; Wayne & Bazzaz 1993; Niinemets 1995, 1996a). Consequently, even if N_m is invariable or decreases, N_a may increase with increasing irradiance owing to the multiplicative effects of light on leaf anatomy (Ellsworth & Reich 1993; Kull & Niinemets 1993; Niinemets 1995, 1996b). Unfortunately, most research on the influence of light on nitrogen partitioning has been conducted with N_a , which confounds the variation in both N_m and LMA, and, therefore, may not serve as an estimate of nitrogen investments for foliage construction (Givnish 1988).

Generally, shade-tolerant species have proportionally higher nitrogen investment in compounds responsible for light capture, but shade-intolerant species invest more in CO₂-carboxylating enzymes (Seemann *et al.* 1987; Evans 1989). Given the saturating nature of the leaf absorptance vs chlorophyll relationship (Evans & Seemann 1989) and the low affinity of the major Calvin cycle enzyme ribulose biphosphate carboxylase/oxygenase to its substrate CO₂ (Badger & Collatz 1977), both efficient quantum capture and high carboxylation capacity are expensive in terms of leaf nitrogen. Therefore, a single species is probably not able to optimize both of them, and a trade-off may occur between the capacities for light harvesting and CO₂ assimilation. Because higher light harvesting and carboxylating capacities are advantageous only at low and high irradiance, respectively, interspecific differences in nitrogen partitioning may provide a mechanistic basis for species separation along light gradients. Insofar as all listed taxa, where N_m responded positively to decreased irradiance, are rela-

tively shade tolerant, and all other species relatively intolerant, different patterns of N_m distribution across natural light gradients seem to be tightly linked to species potential to endure shade.

With this paper I ask: (1) what is the role of inter-specific variability in foliar nitrogen distribution in shade tolerance and (2) is it possible to determine species' shade tolerance on the basis of how foliar nitrogen is partitioned between the leaves and within the leaf across the gradients of irradiance? To gain insight into these issues I studied modifications in leaf nitrogen concentration and in foliar morphology in correlation with changes in relative irradiance in four temperate deciduous woody species of differing known response to shade. To further reinforce the argument, the analysis was extended by introducing a number of other species from several independent studies, where species performance in terms of foliar structure and nitrogen economy has been followed over a wide gradient of relative irradiances along the gap-understorey continuum.

Materials and methods

EXPERIMENTAL SITE AND PLANT SAMPLING

The study was conducted in the understorey of a forest dominated by about 100-year-old trees of *Picea abies* (L.) Karst. on gleyic and gleyed pseudopodzolic soils at Tõrvandi (58° 20' N, 26° 42' E; elevation 60–65 m above sea level), Estonia, in the middle of August 1993. In addition to *P. abies*, trees of *Betula pendula* Roth., *Pinus sylvestris* L., *Populus tremula* L. and *Salix caprea* L. were found in the overstorey, whereas the understorey was dominated by *Populus tremula*, *Padus avium* Miller, *Salix myrsinifolia* Sal. and *Vaccinium myrtillus* L. Saplings of four deciduous woody species were sampled. The saplings of *P. tremula* and *P. avium*, although growing solitarily, were mostly of sprout origin, whereas *A. platanoides* and *Q. robur* were exclusively of seed origin. According to Ellenberg *et al.* (1991), *A. platanoides* is the most and *Q. robur* the least shade tolerant of the species (Table 1). Mean age, estimated by counting the growth rings at the ground, was not significantly different between *Q. robur* and *A. platanoides* (Table 1). Although the plants in more open habitats were usually larger for any particular age, mean height of sampled saplings differed little between the species (Table 1). Species studied at these latitudes usually have determinate growth, annually producing only one flush of leaves. Few plants of *P. tremula* and *Q. robur*, which had flushed twice, were discarded from the analysis. Fully expanded, undamaged leaves were sampled from the uppermost 10-cm crown layer only. Sampling was restricted to midday hours between 12.00 and 13.00 h on cloudy days. Five to nine leaves per sample were analysed and means calculated.

Several hemispheric photographs were made at the

Table 1. Characteristics of sample plants

Species (no. of trees)	Ellenberg's light figure* (% range)	Age (years)		Height (m)		Leaf size (cm ²)
		Range	Mean ± SE [†]	Range	Mean ± SE	Mean ± SE
<i>Acer platanoides</i> (22)	4 (5–10)	4–21	10.2 ± 0.9 ^a	0.30–2.00	1.04 ± 0.11 ^{ab}	62.1 ± 6.1 ^a
<i>Padus avium</i> (28)	5 (10–20)			0.50–1.80	1.28 ± 0.09 ^b	32.2 ± 2.1 ^b
<i>Populus tremula</i> (26)	6 (20–30)			0.54–1.60	1.08 ± 0.06 ^{ab}	38.3 ± 4.2 ^b
<i>Quercus robur</i> (27)	7 (30–40)	3–15	9.1 ± 0.6 ^a	0.30–1.53	0.93 ± 0.06 ^a	34.0 ± 2.4 ^b

* European woody species range according to light figures, which have originally been obtained from observations of species dispersal across gap–understorey gradient, from 3 (most shade tolerant) to 9 (most intolerant) (Ellenberg *et al.* 1991). Species % range, respective to different light figures, was compiled based on the explanations in Ellenberg *et al.* (1991, p. 67).

† Parameters with the same letter are not significantly different (Bonferroni test, $P > 0.05$).

location of sampled leaves and the differences in relative irradiance between the samples were quantified after Anderson (1964) as modified by Nilson & Ross (1979). From every photograph the area of canopy gaps was measured with respect to zenith angle, corrected for cosine of incidence effects and diffuse site factor (RI, relative amount of penetrating diffuse solar irradiance) was calculated for uniformly overcast sky conditions. RI ranges from 0 to 1: a value equal to 1.0 corresponds to the diffuse irradiance above the stand and that of 0.0 to completely closed overstorey canopy with no penetrating gaps. Experimental comparisons confirm that RI gives good estimates to other light sensors in the long term (Salminen *et al.* 1983).

ANALYSIS OF LEAF CHEMICAL AND MORPHOLOGICAL PARAMETERS

Leaf contours were traced manually with a computer digitizer (QD-1212, QTronix Corp., Taiwan) and leaf area was estimated using locally generated software. Leaves were weighed after being oven-dried at 70 °C for at least 48 h. Leaf nitrogen concentration (N_m) was estimated by the standard Kjeldahl method. The content of non-structural carbohydrates (NSC) was determined colorimetrically by the anthrone reaction (Yemm & Willis 1954) as described previously (Niinemets 1995, 1997). Total non-structural carbohydrate content (TNC) was found as the sum of ethanol-soluble carbohydrates (ESC) and starch. Leaf chlorophyll content was estimated in a solution of 96% ethanol with spectrophotometer SF-16 (Lomo, Leningrad, Russia) from the extinctions at 665 and 649 nm minus the extinction at 750 nm (Wintermans & de Mots 1965; Wintermans 1969). Leaves were extracted in the presence of magnesium carbonate to avoid the conversion of chlorophylls to pheophytins.

Because diurnal variability in irradiance strongly affects leaf photosynthesis (Servaites *et al.* 1989), but effects carbon translocation from the leaves much less (Hendrix & Huber 1986), concentration of foliar non-structural carbohydrates exhibits the highest daily fluctuation of leaf substances (Plhák 1984). This variability significantly influences LMA (Chatterton, Lee

& Hungerford 1972) and the concentration of other leaf compounds (Servaites *et al.* 1989). Moreover, cumulative photosynthetic production and the content of NSC are also related (Takahashi, Tsuchihashi & Nakaseko 1993), resulting in increasing pool size of non-structural carbon with increasing relative irradiance (Niinemets 1995, 1997). Consequently, in many cases the conclusions are safer if they are based on the leaf parameters calculated on a NSC-free dry mass. This procedure follows Niinemets (1995, 1997): LMA was multiplied, and N_m and leaf chlorophyll concentration (Chl_m) were divided by 1-TNC (TNC is expressed here as the proportion of leaf dry mass) to get leaf NSC-free dry mass per area (LMA^c), and the concentrations of nitrogen (N_m^c) and chlorophyll (Chl_m^c) in NSC-free dry mass.

Linear correlation, linear and non-linear regression techniques were used for analysing the data. Differences between the means were tested by one-way ANOVA, and in statistical relationships by one-way ANCOVA. The Bonferroni test was used to separate the treatment means. All relationships were considered significant at $P < 0.05$ (Wilkinson 1990).

Results

LEAF MORPHOLOGY, NON-STRUCTURAL CARBOHYDRATES

The leaves were of similar size in *P. avium*, *P. tremula* and *Q. robur*, but significantly larger in *A. platanoides* ($P < 0.001$, Table 1). In all species, both LMA and LMA^c increased significantly with increasing relative irradiance (RI) (Fig. 1). The LMA vs RI relationship was generally linear, slightly curving only in *P. tremula*. The dependence of LMA^c on RI was linear for all species. The slopes of LMA and LMA^c vs RI relations were lowest in *A. platanoides*, intermediate in *P. tremula* and *Q. robur*, and highest in *P. avium*. Subtraction of non-structural carbohydrate content from total leaf dry mass always resulted in lower slopes of the relationship between LMA^c and RI than of LMA vs RI, because there was a tendency for the concentration of total non-structural carbohydrates (TNC, ethanol-soluble carbohydrates plus starch) to

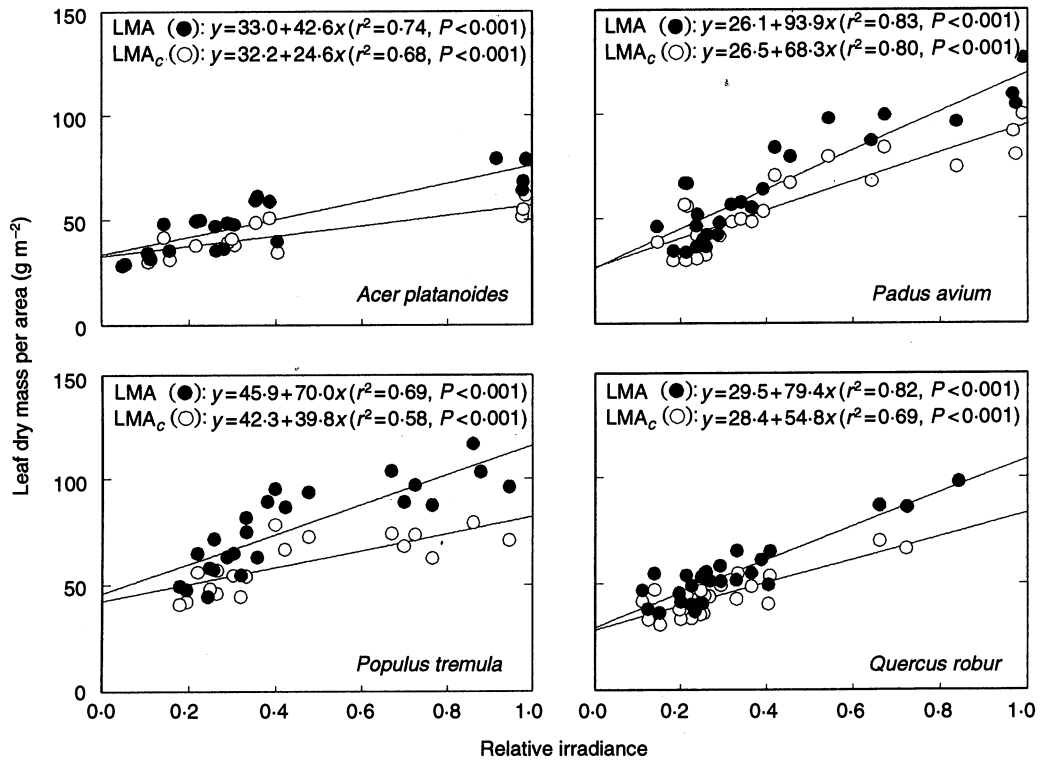


Fig. 1. Dependence of total leaf dry mass per area (LMA, g m⁻²) and non-structural carbohydrate free dry mass per area (LMA_c, g m⁻²) on relative irradiance.

increase with LMA in all species ($P<0.05$ for *A. platanoides* and $P<0.001$ for other species) and with RI in all, except for *A. platanoides* (Fig. 2). Inasmuch as starch content was positively related to RI only in *P.*

avium ($r^2=0.19$, $P<0.05$), the correlations between RI and TNC, and between LMA and TNC were largely attributable to an increase in ethanol-soluble carbohydrates (mostly oligo- and monosaccharides)

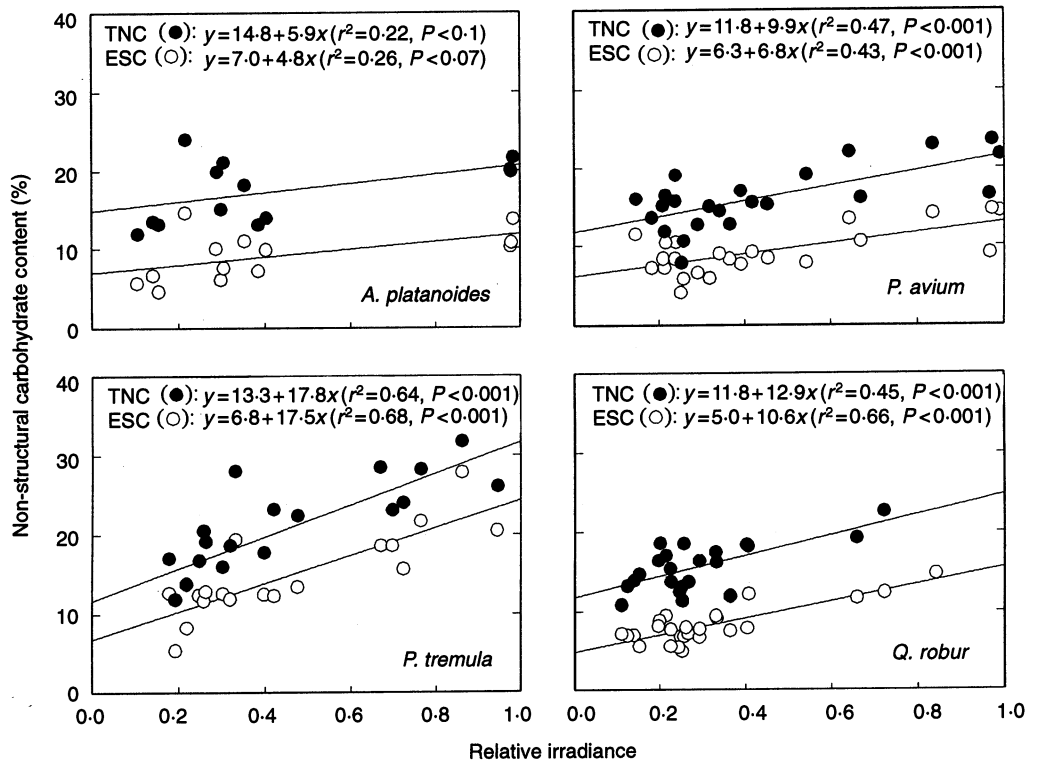


Fig. 2. Pool size of leaf non-structural carbohydrates (%) in dependence on irradiance. ESC: ethanol-soluble carbohydrates. TNC: ESC plus starch.

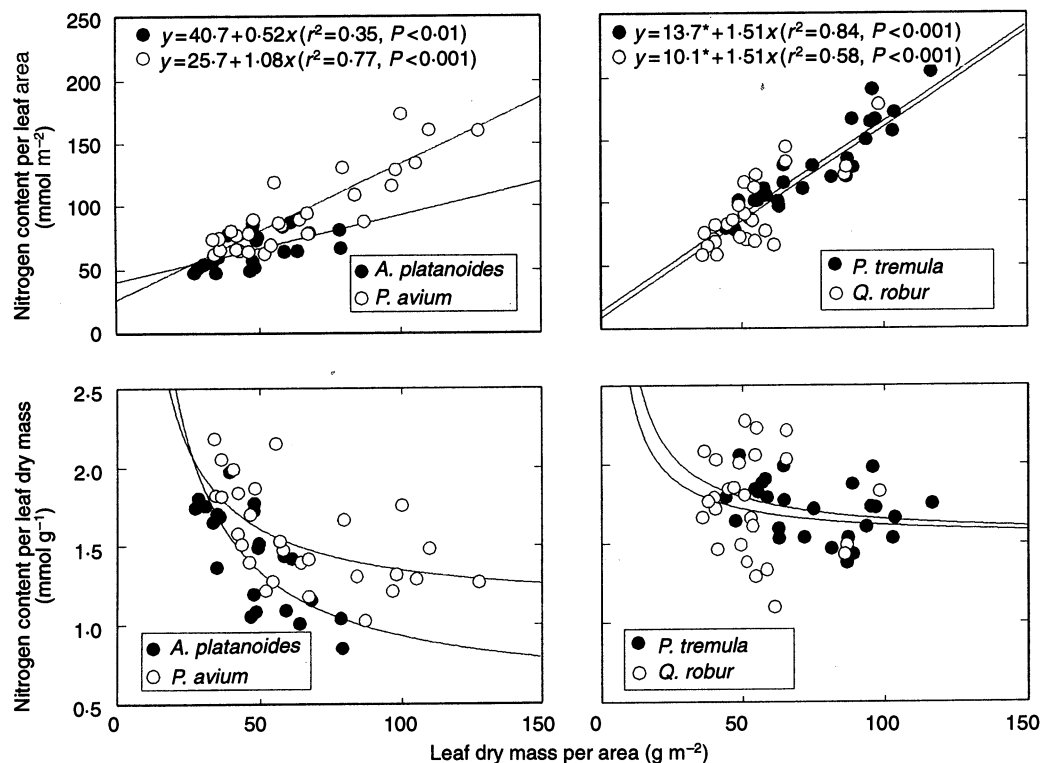


Fig. 3. Relationships between leaf area (N_a , mmol m^{-2}) and dry mass-based (N_m , mmol g^{-1}) nitrogen content, and LMA. The curves on N_m vs LMA panels are derived from regression coefficients of linear N_a vs LMA relationships ($N_a = a \times \text{LMA} + b$, $N_m = a + b/\text{LMA}$). Intercepts denoted by * are not significantly different from zero ($P > 0.2$).

with RI and LMA (Fig. 2). Mean starch content was not significantly different between the species ($P > 0.1$). Owing to the constancy of starch concentration, the slopes of the ESC vs RI and TNC vs RI relationships were similar within a species (Fig. 2). However, the slopes differed between the species: according to the slope of ESC vs RI relationship the species ranked as *A. platanoides* < *P. avium* < *Q. robur* < *P. tremula*.

PARTITIONING OF LEAF CHLOROPHYLL AND NITROGEN

N_a was positively related to LMA in all species (Fig. 3) but the correlation with LMA^c (Table 2) and RI ($r^2 = 0.15$, $P > 0.07$) was not significant for *A. platanoides*. In other species, N_a was slightly less significantly correlated with RI (r^2 ranged from 0.48 to 0.73,

$P < 0.001$) than with LMA (Fig. 3) and LMA^c (Table 2). In *A. platanoides* and *P. avium* N_m decreased with LMA (Fig. 3), LMA^c and RI (data not shown), but it was independent of these variables in *P. tremula* and *Q. robur*. N_m^c (N_m per NSC-free dry mass) behaved similarly to N_m . However, there were two exceptions: N_m^c increased with RI in *P. tremula* ($r^2 = 0.22$, $P < 0.05$) and was not significantly related to RI in *P. avium* ($r^2 = 0.10$, $P > 0.1$).

Leaf chlorophyll content per leaf area (Chl_a) was independent of irradiance in all species ($P > 0.2$), except for *A. platanoides*, where it was negatively correlated with RI ($r^2 = 0.26$, $P < 0.02$, respectively). However, chlorophyll content per dry mass (Chl_m , Chl_a/LMA) increased with decreasing RI (Fig. 4) and LMA ($r^2 = 0.38\text{--}0.75$, $P < 0.001$) in all species. Similar correlations also occurred with Chl_m^c (for RI:

Table 2. Relationships between leaf nitrogen content per leaf area (N_a) and leaf non-structural carbohydrate (NSC, ethanol-soluble carbohydrates plus starch) free dry mass per area (LMA^c), and between leaf chlorophyll (a+b) (Chl_m^c) and nitrogen contents per NSC-free dry mass (N_m^c)

Species	N_a (mmol m^{-2}) vs LMA^c (g m^{-2})					Chl_m^c ($\mu\text{mol g}^{-1}$) vs N_m^c (mmol g^{-1})					n
	Intercept	P	Slope	P	r^2	Intercept	P	Slope	P	r^2	
<i>Acer platanoides</i>	45.3	0.01	0.59	0.1	0.24	-19.5	0.001	18.4	0.001	0.84	13
<i>Padus avium</i>	16.6	0.08	1.47	0.001	0.81	-14.2	0.01	13.3	0.001	0.53	24
<i>Populus tremula</i>	-10.5	0.52	2.42	0.001	0.83	7.8	0.21	1.4	0.56	0.02	20
<i>Quercus robur</i>	0.8	0.99	2.03	0.001	0.66	-7.7	0.17	9.4	0.002	0.42	22

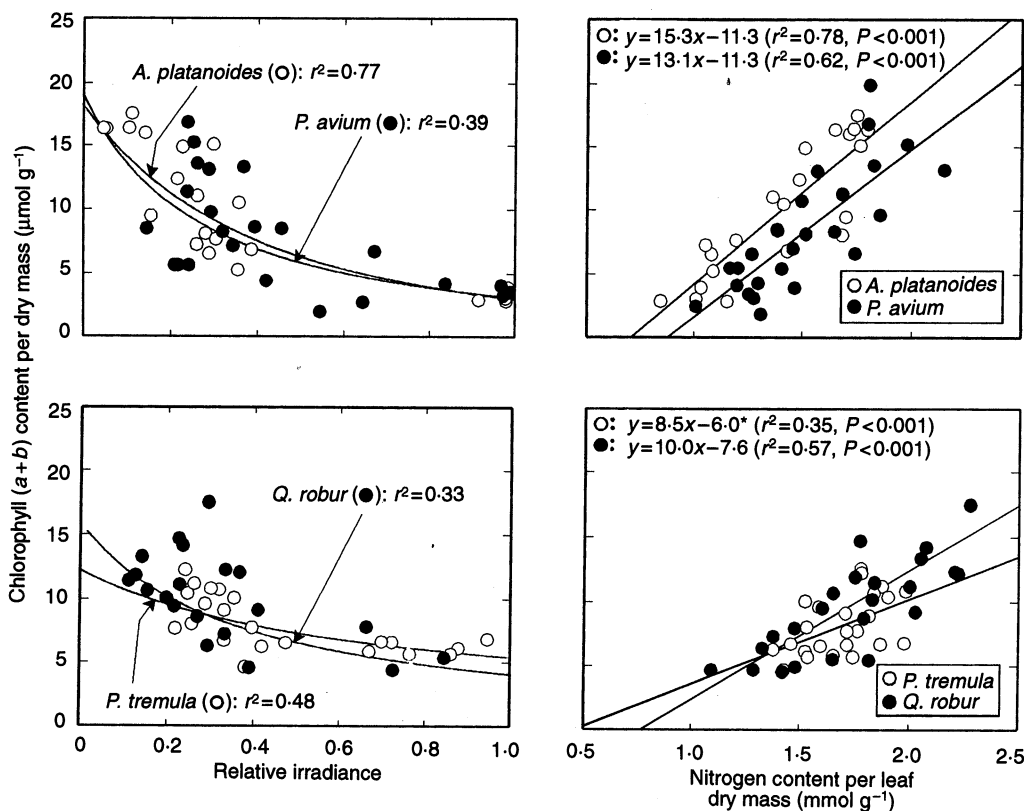


Fig. 4. Effect of relative irradiance and leaf nitrogen concentration (N_m) on leaf chlorophyll (a + b) content per total leaf dry mass (Chl_m , $\mu\text{mol g}^{-1}$). Hyperbolas, in the form of $Chl_m = a + b/RI$, were fitted to the data by non-linear regression. *, intercept is not significantly different from zero ($P > 0.3$).

$r^2 = 0.23\text{--}0.41$, $P < 0.05$; for LMA^c: $r^2 = 0.27\text{--}0.58$, $P < 0.02$). Relationships of Chl_m vs RI (Fig. 4) and Chl_m vs LMA (data not shown) were not linear. Hyperbolic non-linear fits resulted in a higher proportion of determined variance than the linear approximations (e.g. for Chl_m vs RI relations the r^2 range was 0.33–0.77 for the hyperbolic vs 0.29–0.43 for the linear fit).

Chl_a increased with N_a only in *Q. robur* ($r^2 = 0.26$, $P < 0.02$), but Chl_m was significantly related to N_m in all species (Fig. 4). As multiple regression analysis revealed, this relationship was not just driven by low RI data, where N_m in *A. platanoides* and *P. avium* (Fig. 3), and Chl_m in all species (Fig. 4) were the highest, because N_m always remained significant ($P < 0.001$) when the variability in RI was accounted for. However, the correlation between the quantities expressed on a NSC-free dry mass was not significant for *P. tremula* (Table 2). Furthermore, it appeared that Chl_m increased faster with decreasing irradiance than N_m , causing the ratio of leaf nitrogen to chlorophyll (N/Chl) to be positively related to RI in all species (Fig. 5). Insofar as both N_m at high irradiance (Fig. 3) and the intercepts of N/Chl vs RI relationship (Fig. 5) were the least, and the slopes of Chl_m vs N_m the highest (Fig. 4) in shade-tolerant *A. platanoides* and *P. avium*, the fraction of leaf nitrogen associated with leaf chlorophyll is likely to be higher in shade-tolerant

species than in intolerant species. Taking $40 \text{ mol N mol}^{-1}$ Chl for the nitrogen cost of light harvesting (Evans 1989), the proportion of leaf N invested in light harvesting behaved similarly to Chl_m increasing hyperbolically with decreasing irradiance (data not shown). Though the qualitative tendency was identical for all species, the range of variation was higher in more shade-tolerant *A. platanoides* (8–40%) and *P. avium* (8–50%) than in less tolerant *P. tremula* (12–30%) and *Q. robur* (12–30%). Chl a/b ratio was relatively insensitive to irradiance (Fig. 5), declining significantly with decreasing RI only in *P. avium* and *P. tremula*, and with LMA in *P. avium* ($r^2 = 0.20$, $P < 0.03$) and *Q. robur* ($r^2 = 0.19$, $P < 0.05$).

IMPLICATIONS FOR SHADE TOLERANCE

The intercept of N_a vs leaf dry mass per area relationship was not statistically different from zero in *P. tremula* and *Q. robur* (Fig. 3, Table 2). Nevertheless, a gradual decline in the intercept values from *A. platanoides* to *Q. robur*, that is, from the most shade-tolerant to most intolerant species (Table 1), was accompanied by decreasing steepness of the N_m vs LMA relations (Fig. 3). This gave a significant negative correlation between the intercept of N_a vs LMA and Ellenberg's light figure (LF, see Table 1 for explana-

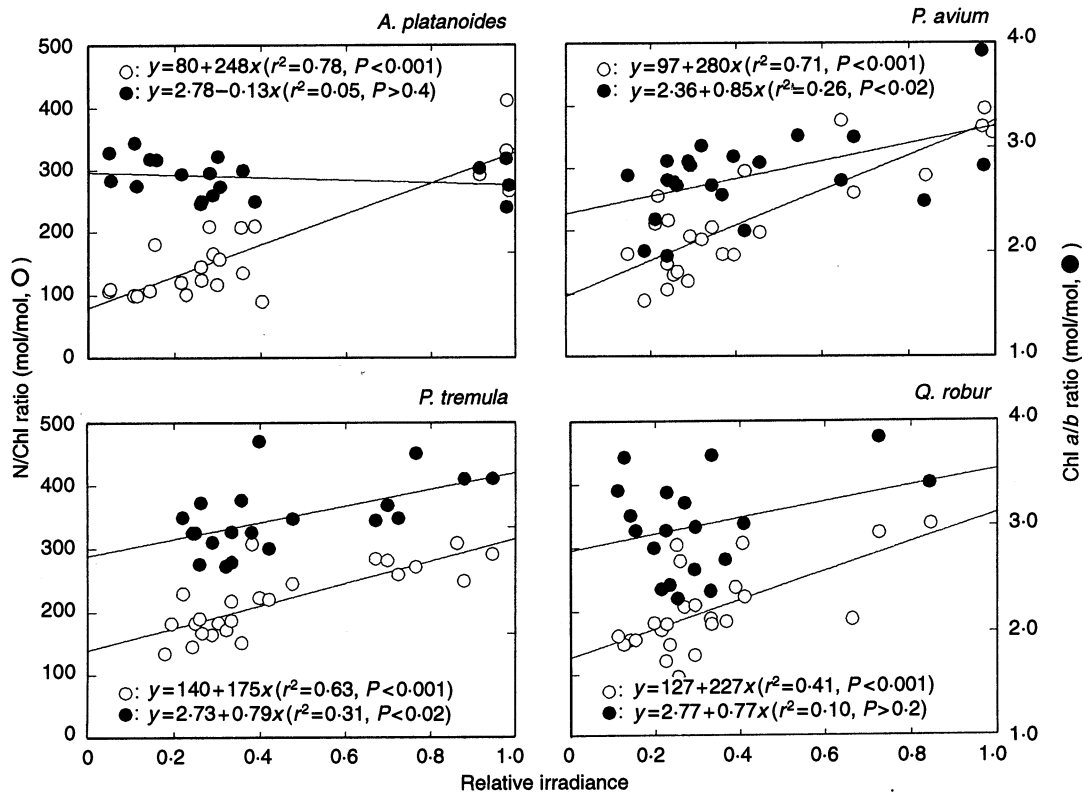


Fig. 5. Influence of relative irradiance on the molar ratios of leaf nitrogen to chlorophyll (a + b) (N/Chl) and chlorophylls a to b (Chl a/b).

tion) ($r^2 = 0.94$, $P < 0.03$). Though LF was not significantly related to the slope of N_a vs LMA in the studied species ($r^2 = 0.87$, $P < 0.07$), a good negative correlation was present between the slope and the intercept values ($r^2 = 0.98$, $P < 0.001$). This correlation was even improved when the confounding variability in NSC was accounted for ($r^2 = 0.999$, $P < 0.001$ for the correlation between the intercept and slope of N_a vs LMA^c). When further data for the relationships of N_a vs LMA for deciduous species *B. pendula*, *C. avellana* and *L. xylosteum* (Kull & Niinemets 1993), and evergreens *P. abies* (recalculated from Benecke 1982; Matyssek 1985; Mikkelsen, Dodell & Lütz 1995, $n = 7$, $r^2 = 0.54$, $P < 0.05$) and *P. sylvestris* (recalculated from Linder 1972, 1980; Matyssek 1985; Schulze & Küppers 1985; $n = 9$, $r^2 = 0.74$, $P < 0.005$) were pooled with those of the current study, both the slope (Fig. 6a) and intercept ($r^2 = 0.61$, $P < 0.01$) were significantly related to LF. The evergreen conifers had lower nitrogen concentrations than deciduous species (data not shown), and removal of these species from the analysis of the relationships between LF and coefficients of N_a vs LMA improved the correlations somewhat (e.g. in the relationship between the slope and LF $r^2 = 0.62$ with and $r^2 = 0.79$ without conifers). Incorporation of several other species, such as temperate *A. saccharum* (Ellsworth & Reich 1992), *Betula alleghaniensis* (recalculated from Wayne & Bazzaz 1993; $n = 8$, $r^2 = 0.08$, $P < 0.001$) and *Betula populifolia* (recal-

lated from Wayne & Bazzaz 1993; $n = 8$, $r^2 = 0.70$, $P < 0.01$), subtropical *P. persica* (DeJong & Doyle 1985) and tropical *Borojoa patinoi* (recalculated from Lynch & González 1993; $n = 17$, $r^2 = 0.72$, $P < 0.001$), for which no shade-tolerance scale comparable to Ellenberg's one was available, into the analysis, showed that this relationship (Fig. 6b) is robust and does not depend on leaf longevity.

To gain further insight into the interrelationships between nitrogen partitioning among the leaves and habitats (Fig. 6a,b) and within the leaf, the parameters of the relationships of leaf chlorophyll vs nitrogen and irradiance were examined for the dependence on the parameters of the N_a vs LMA relationship. In addition to the data depicted on Figs 4 and 5, N/Chl vs RI and Chl_m vs N_m relationships were derived for six other species (Fig. 6c). It appeared that the proportional investment of leaf nitrogen in chlorophyll at low light (the intercept of N/Chl vs RI) was positively related to the slope of N_a vs LMA (Fig. 6c) as well as to LF (only European species; $r^2 = 0.83$, $P < 0.05$), and negatively to the intercept of N_a vs LMA ($r^2 = 0.64$, $P < 0.005$). The slope of Chl_m vs N_m , characterizing the responsiveness of Chl_m to changes in nitrogen availability, was independent of the slope of N_a vs LMA ($r^2 = 0.10$, $P > 0.3$); however, it increased with increasing the intercept of N_a vs LMA ($r^2 = 0.42$, $P < 0.05$) and was negatively correlated with the intercept of N/Chl vs RI ($r^2 = 0.50$, $P < 0.05$). The latter correlations imply that improved nitrogen nutrition

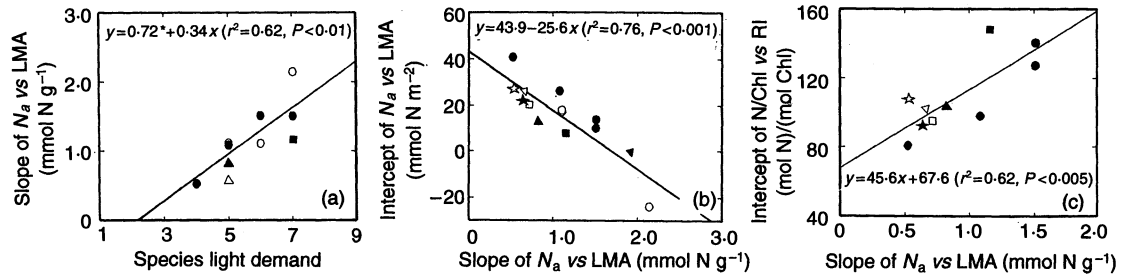


Fig. 6. Nitrogen distribution in relation to leaf dry mass per area vs species light requirement. (a) Dependence of the slope of the N_a vs LMA relationship (mmol N g⁻¹) on species light demand value. Regression is based on the data of the current study (●), and on *Betula pendula*, *Corylus avellana* and *Lonicera xylosteum* (○, Kull & Niinemets 1993), *Picea abies* [▲, 0 and 1-year-old needles, mid-season; recalculated from Matyssek (1985); Mikkelsen *et al.* (1995) and Benecke (1972) using the relationships between needle structure and irradiance of Niinemets (1997)] and *Pinus sylvestris* [■, 0 and 1-year-old needles, mid-season; recalculated from Schulze & Küppers (1985) (Mg-deficient trees omitted from the analysis), Linder (1972, 1980) and Matyssek (1985) using the relationships between needle structure and irradiance of Kellomäki & Oker-Blom (1981) and Zhelawski, Kinelska & Lotocki (1968)]. For comparison another value for *P. abies* (△, 0-year-old needles) from Niinemets (1996b) has also depicted. The light demand scale is that of Ellenberg (1991, see also Table 1). *, intercept is not significantly different from zero ($P > 0.2$). (b) Correlation between the intercept (mmol N m⁻²) and the slope of the N_a vs LMA relationship. In addition to the European species plotted in (a), *Acer saccharum* (□, Ellsworth & Reich 1992), *Betula alleghaniensis* and *Betula populifolia* (★ and ☆, respectively, recalculated from Wayne & Bazzaz 1993; only 'gap' samples), *Borojoa patinoi* (▷, Lynch & González 1993) and *Prunus persica* (►, DeJong & Doyle 1985) have also been included in the regression. (c) Relationship between the intercept of N/Chl vs RI [(mol N)/(mol Chl)] and the slope of N_a vs LMA. Data presentation as in (b), except that in addition to Ellsworth & Reich (1992), the data of Ellsworth & Reich (1993) and Tjoelker *et al.* (1995) have been used to develop the relationship between N/Chl vs RI in *A. saccharum* ($r^2 = 0.50$, $P < 0.05$).

enhances leaf chlorophyll concentration primarily in shade-tolerant species at low light.

Discussion

LEAF MORPHOLOGY

Because LMA and RI are linearly related (Fig. 1 and the references in the Introduction), LMA, which integrates the physiological effects of different light quantities, qualities (Kwesiga, Grace & Sandford 1986) and peak intensities (Wayne & Bazzaz 1993) on leaf structure, may be an even better estimate of long-term light climate than RI. However, species differ remarkably in the tuning of leaf morphology with respect to the prevailing irradiance. In general, at a common irradiance, shade-tolerant species have lower LMA than shade-intolerant ones (Niinemets & Kull 1994, but see Niinemets 1996b). However, there exists a large interspecific variability in morphological flexibility with respect to light. On the basis of LMA vs RI relationships, shade-tolerant species may have greater (Goulet & Bellefleur 1986; Kull & Niinemets 1993; and *P. avium* in this study) or lower morphological flexibility (Loach 1967; Fetcher, Strain & Oberbauer 1983; Walters & Field 1987; Ducrey 1992; and *A. platanoides* in this study); and on the basis of leaf thickness vs RI, greater (Goulet & Bellefleur 1986; Chazdon & Kaufmann 1993), similar (Carpenter & Smith 1981) or lower (Jackson 1967; Fetcher *et al.* 1983; Ashton & Berlyn 1994) plasticity than intolerant species. In the current study, the lowest morphological plasticity in *A. platanoides* may be related to the large leaf size in this species (Table 1). Because the costs for

leaf support scale positively with leaf size (Givnish 1979), a trade-off goes on between light-mediated anatomical adjustment in leaf thickness and rapidly increasing carbon demand for leaf support. Nevertheless, the current study and the aforementioned evidence from many independent works seem to support the argument that the considerations of species plasticity based on leaf anatomy and morphology alone do not lead to generalizations that would hold for a large number of taxa.

NON-STRUCTURAL CARBOHYDRATES

Because photosynthesis strongly depends on irradiance, but carbon translocation is nearly constant (Servaites *et al.* 1989) or only slightly light dependent (Hendrix & Huber 1986), the concentration of leaf non-structural carbohydrates closely follows diurnal course of light (Servaites *et al.* 1989). Accordingly, higher daily cumulative photosynthesis at greater irradiances may be proposed as an explanation for the positive relationships between ESC and TNC with RI (Fig. 2; Niinemets 1995, 1997). Of course, species potentials in terms of photosynthetic capacity may also affect the non-structural carbohydrate vs irradiance relations (Takahashi *et al.* 1993). Photosynthetic capacity per leaf area (P^a_{max} , light-saturated net photosynthesis rate at an ambient CO₂ concentration of c. 330 p.p.m.) for leaves grown at a RI of 0.9 is 9.0 for *P. tremula* (Tselniker 1979), 7.3 for *Q. robur* (Malkina 1983) and 5.1 μmol m⁻² s⁻¹ for *A. platanoides* (Tselniker 1979). Calculation of P^m_{max} (photosynthetic capacity per leaf dry mass, P^a_{max}/LMA) from the LMA

values at $RI=0.9$ (Fig. 1) gives a species ranking, which is the same as according to the slope values of ESC vs RI relationships. This calculation shows that the confounding effect of ESC on diverse leaf chemical and morphological parameters is variable at a common irradiance between the species owing to different P_{max}^m values. To exclude the light- and species-related variation in ESC, expression of leaf parameters on a NSC-free dry mass may serve as a useful alternative to total dry mass. Yet the subtraction of NSC from total dry mass is open to criticism, because (1) a part of leaf ESC, such as leaf glycosides (which also give colour reaction with anthrone, Yemm & Willis 1954), may not be directly associated with daily photosynthetic carbon metabolism as becomes evident from the non-zero intercept values of ESC vs RI relations (Fig. 2) and (2) the NSC pool is not metabolically inactive, e.g. accumulation of NSC may suppress further photosynthesis (Azcón-Bieto 1983). Nevertheless, in the current study the conclusions were robust and were only slightly biased by using the parameters per total rather than per NSC-free dry mass.

PARTITIONING OF LEAF CHLOROPHYLL AND NITROGEN: SIGNIFICANCE FOR LIGHT HARVESTING

An increase in the proportional investment of leaf nitrogen in thylakoids, improving incident light-use efficiency, is a major response of nitrogen partitioning within the leaf to decreased growth irradiance (Evans 1989, 1993a). There is a wide consensus that this increase is even more significant for species of greater shade tolerance (Seemann *et al.* 1987; Evans 1989, 1993a). These previously established patterns were also observed in the current study. While N_m varied about twofold with RI and LMA (Fig. 3), the differences in Chl_m were up to fivefold (Fig. 4), resulting in a positive relationship between N/Chl and RI (Fig. 5), and in a negative one between the nitrogen investment in light harvesting and RI. Of course, calculation of the nitrogen cost of light harvesting using a constant conversion factor of $40 \text{ mol N mol}^{-1} \text{ Chl}$ (Evans 1989) over the whole range of RI is a simplification, and may have resulted in biased estimates, because the light-related modification of thylakoid composition influences the nitrogen requirement for thylakoid formation as well (Evans & Seemann 1989). Adaptation to low irradiance involves an increase in light-harvesting complexes of photosystem II (LHC II) (Leong & Anderson 1984; Anderson & Osmond 1987), which are cheaper in terms of nitrogen per unit of chlorophyll than, for example, the protein complexes of photosystems I and II (Evans & Seemann 1989). Given that LHC II proportionally increases with decreasing $Chl\ a/b$ ratio (Anderson & Osmond 1987; Evans 1988), and that $Chl\ a/b$ ratio was weakly related to RI in the current study (Fig. 5), the qualitative relationships between nitrogen investment for light harvesting

and irradiance are not likely to be altered by ignoring the effects of light on thylakoid composition.

It is not clear why the relationships between $Chl\ a/b$ ratio and RI were poor, but there is evidence that $Chl\ a/b$ ratio may depend on light quality rather than quantity (Liu, Chow & Anderson 1993). Because light quality in the understorey is primarily dependent on floristic composition of the overstorey (Holmes & Smith 1977), red/far red ratio, a characteristic of differences in light quality in vegetation canopies, varies more than twofold at a common light quantity in stands dominated by different species (St-Jacques & Bellefleur 1993). This appears relevant for the interpretation of the results of the current study and I suggest that heterogeneous spectral composition of light at common RI in various stand regions, where different taxa were major canopy species, may have brought about loose relationships between $Chl\ a/b$ ratio and RI (Fig. 5). Similarly, Bazzaz & Wayne (1994), who studied 18 different traits in four species of *Betula* in three experimental light treatments, concluded that while Chl_m is one of the most plastic, $Chl\ a/b$ ratio is the least plastic of the studied traits.

Because the relationship between Chl_a and leaf absorptance is asymptotic (Gabrielsen 1948; Evans & Seemann 1989; Agustí *et al.* 1994), enhanced quantum capture is expensive in units of nitrogen and large investments of leaf nitrogen in light-harvesting compounds result in only moderately improved returns in terms of intercepted light (Evans & Seemann 1989). With decreasing irradiance LMA decreases (Fig. 1) and progressively more nitrogen should be invested in light-harvesting compounds ($Chl_a = LMA \times Chl_m$) to achieve comparable or higher absorptance relative to the leaves grown in high light. This is probably why N_m increases with decreasing irradiance (Fig. 3). The explanation of higher N_m at low irradiance in shade-tolerant species by disproportionately increasing nitrogen requirements for light-harvesting chemicals is further supported by the more responsive N_m in shade-tolerant species (Fig. 3, Table 2), which also have a greater fraction of foliar resources in light-intercepting compounds (cf. above).

Dependence of light absorptance of plant tissues in photosynthetically active radiation (PAR, 400–700 nm, A_l) on their chlorophyll content has a fundamental character (Agustí *et al.* 1994), which is similar to all plant taxonomic groups. In conditions where reduced carbon incomes owing to low light levels seriously restrict leaf area production, or in tree species where leaf growth patterns are less flexible owing to determinate growth (e.g. the species in the current study) light limitation imposed on photosynthesis may be relieved by increasing leaf chlorophyll concentration, which improves A_l and the quantum yield for an incident light flux (e.g. Gabrielsen 1948). However, this is different at greater irradiances, where high chlorophyll content causes also mutual shading between the photosynthesizing cells in a leaf to

increase and thereby decrease photosynthesis at intermediate and high light (Leverenz 1987), i.e. P_{\max}^a shifts to unrealistically high irradiances, where increased photoinhibition impedes the realization of biochemical capacities of leaf cells (Evans, Jakobsen & Ögren 1993). Therefore, for optimum photosynthetic performance, leaf absorptance should be high under low irradiance and low under high irradiance (Laisk 1982). However, the data which support this hypothesis are contradictory. A_l is higher in low than in high light environments in several temperate and tropical woody species (Langenheim *et al.* 1984, St-Jacques, Labrecque & Bellefleur 1991; Thompson, Huang & Kriedemann 1992; Poorter, Oberbauer & Clark 1995), but is independent of growth irradiance in *Minuartia guianensis* (Poorter, Oberbauer & Clark 1995), *Pisum sativum* (Evans 1987) and *Toona australis* (Thompson *et al.* 1992), and decreases with decreasing irradiance in *F. sylvatica* (Eller *et al.* 1981), *Nothofagus solandri* (Hollinger 1989) and *Simarouba amara* (Poorter *et al.* 1995). Leaf transmittance, which is negatively related to A_l (Osborne & Raven 1986), is independent of growth irradiance in seedlings of five temperate species (Tselniker 1975). Because A_l is a function of Chl_a (Evans 1993b; Agustí *et al.* 1994), the inconsistency in the reactions of A_l to light availability should be paralleled with similar variability in Chl_a vs irradiance relationships. This is indeed the case: with decreasing irradiance Chl_a increases in *A. platanoides* (this study), *A. saccharum* (Ellsworth & Reich 1992), *Fagus grandifolia* (Loach 1967), *Lea coccinia* (Sarracino, Merritt & Chin 1992) and in a number of tropical woody species (Langenheim *et al.* 1984; Thompson *et al.* 1992), decreases in *B. pendula* (Öquist, Brunet & Hällgren 1982), *Canarina canariensis* (Morales, Jimenez & Caballero 1991), *Eucalyptus fastigata* (Cameron 1970), *F. sylvatica* (Eller, Glättli & Flach 1981; Lichtenthaler *et al.* 1981) or is virtually constant in *Borojoa patinoi* (Lynch & González 1993), *Dipteryx panamensis* and *Lecythis ampla* (Poorter *et al.* 1995), *P. avium* and *P. tremula* (this study) and *Populus tremuloides* (Loach 1967). Examination of mass-based chlorophyll content demonstrates that the constancy of Chl_a across light gradient is achieved by large investments of foliar resources in chlorophyll: Chl_m consistently increases with decreasing growth irradiance (Fig. 4; Loach 1967; Eller *et al.* 1981; Lichtenthaler *et al.* 1981; Wilhelm & Wild 1984; Morales *et al.* 1991; Dale & Causton 1992; Lynch & González 1993; Poorter *et al.* 1995). According to Wilhelm & Wild (1984) and the current study (Fig. 4) this response is non-linear.

Leaf anatomy is a relevant factor affecting leaf absorptance, because apart from influencing the photon path length in leaves (Osborne & Raven 1986; Vogelmann 1993), it determines Chl_m , which is necessary to attain the same absorptance ($\text{Chl}_a = \text{Chl}_m \times \text{LMA}$). Accordingly, for a common A_l the requirement of leaf resources in light harvesting is

dependent on the morphological plasticity of a species. As the current study (in terms of Chl_a) and previous research (cf. above) demonstrate, A_l and growth irradiance are not necessarily correlated. Nevertheless, leaf absorptance per mass rather than per area provides a measure of how adequately photosynthesizing cells are supplied with light. Inasmuch as a highly significant correlation occurs between Chl_m and leaf absorptance per mass (Agustí *et al.* 1994), adaptation to low irradiance seems to involve modifications in leaf chemistry towards optimizing light interception per mass rather than per area (Fig. 4). As the study of Poorter *et al.* (1995) indicates, efficient light harvesting is less expensive in terms of biomass investment in leaves in understorey, where leaves have higher absorptance per mass, than in leaves in overstorey.

IMPLICATIONS FOR SPECIES SHADE TOLERANCE

Photosynthetic plasticity, the slope of the change in either P_{\max}^m or P_{\max}^a with growth irradiance, is consistently lower in shade-tolerant than in shade-intolerant species (Bazzaz & Carlson 1982; Kwesiga *et al.* 1986; Chazdon & Kaufmann 1993; Ashton & Berlyn 1994; Ducrey 1994). This difference is associated with proportionally lower investment of foliar nitrogen in carboxylating enzymes in shade species (Seemann *et al.* 1987). However, photosynthesis at saturating irradiance poorly reflects photosynthetic behaviour of the species under limiting irradiance, where inverse patterns become evident (Givnish 1988; Koike 1988; Ducrey 1994). In low-light conditions, where foliar morphology and physiology are modified towards efficient use of light, it may clearly not be essential to maximize the rate of light-saturated photosynthesis for a given N_m . There is evidence that the partitioning pattern of foliar nitrogen changes continuously during succession and that interspecific variability in nitrogen distribution may be a relevant feature determining species composition: early successional species have higher nitrogen-use efficiency (P_{\max}^m/N_m) and greater slopes of N_m vs P_{\max}^m relationships than late successional species (Reich *et al.* 1994). As succession goes on, efficiency of light-use gains in importance and species that are not able (intolerant species) to adjust their physiology and morphology towards improved light interception are replaced by those that are (shade-tolerant ones).

Because the slope and intercept of the N_a vs LMA relationship determine how N_m is distributed along a light gradient, significant correlations between these parameters and species light requirement (Fig. 6a) indicate that the distribution pattern of N_m may play a central role in species shade tolerance. Given that the slopes of N_a vs LMA (N_a vs LMA^c) are statistically more reliable than the intercepts (Fig. 3, Table 2), and closely related to intercepts (Fig. 6b), they may serve

as simple predictors of species shade tolerance. Furthermore, the highly significant correlation between the slope of N_a vs LMA, reflecting high photosynthetic capacity at high light, and the intercept of N/Chl vs RI, reflecting high chlorophyll at low light (Fig. 6c), further strengthens the predictive value of N_a vs LMA relationships.

The higher N_m is at low light, the lower is N_m at high light (Fig. 6b). Though the nitrogen requirements for light harvesting decrease with increasing irradiance, efficient use of high irradiances calls for increasing nitrogen investment in carboxylating enzymes and proteins responsible for photosynthetic electron transport. Owing to decreased nitrogen concentrations at high irradiance (Fig. 3), the latter demands are not likely to be met adequately in shade-tolerant species. Conservative nitrogen investment patterns in light harvesting vs carbon reduction capacities probably explains why shade-tolerant species are of intrinsically low photosynthetic plasticity, and accordingly of low competitive ability in high-light environments.

Evergreens display similar responses to deciduous species: N_m increases with decreasing irradiance in shade-tolerant *Abies alba* (Aldinger 1987), *B. patinoi* (Lynch & González 1993) and *P. abies* (Aldinger 1987; Niinemets 1997), but shows the opposite trend in intolerant *Eucalyptus grandis* (Leuning, Cromer & Rance 1991), *Pinus radiata* (Kelly & Lambert 1972), *P. sylvestris* (Heinze & Fiedler 1976) and *Pseudotsuga menziesii* (Lavender & Carmichael 1966). However, conifers tended to have lower slope of N_a vs LMA relationship than the deciduous species at a common light demand (cf. *P. abies* and *P. sylvestris* in Fig. 6a). This discrepancy is probably related to lower foliar nitrogen concentrations and physiological activity in conifers vs deciduous species (Reich, Walters & Ellsworth 1992; Reich *et al.* 1995). Nevertheless, it is striking that a wide range of species including temperate and tropical deciduous and evergreen species fits the basic relationships between shade tolerance and nitrogen partitioning (Fig. 6). Thus, these relationships may form a valuable basis for developing a comparative shade-tolerance scale for species of different geographical locations. Of course, the dependencies between N_a and LMA are affected by site nutrient availability conditions (Walters & Reich 1989). However, nutrient accessibility also alters the minimum light level the species requires for survival, because it changes the photosynthetic light-use efficiency for incident light. This means that, although relative light requirements of species are remarkably conservative among temperate European and North American woody taxa (Niinemets 1996c), no single minimum light level corresponds to different shade-tolerance classes. Thus, quantitative relationships between the parameters of leaf nitrogen partitioning vs irradiance may be particularly useful for determining species absolute light requirements in relation to site-specific factors, such as nitrogen.

From a canopy perspective, lower N_a at a common irradiance in shade-tolerant species (Fig. 6a) results in less costly leaves in terms of nitrogen, and allows construction of a larger foliar surface area with the same investment of plant nitrogen in leaf production. This should lead to a more extensive foliar display for light capture and finally to a significant improvement of light interception per plant. Indeed, previous research has demonstrated that at a common irradiance the saplings of similar size of *A. platanooides* have greater proportion of plant biomass in foliage and also greater foliar surface area than those of *Q. robur* (Niinemets 1996a).

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