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Acclimation to high irradiance in temperate deciduous trees in the field: changes in xanthophyll cycle pool size and in photosynthetic capacity along a canopy light gradient

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ABSTRACT

To test the hypothesis that in temperate deciduous trees acclimation to potentially damaging high irradiances occurs via long-term adjustments in foliar photosynthetic capacity, and short-term changes in xanthophyll cycle pool size in response to weather fluctuations, nitrogen concentration and pigment composition were examined along a canopy light gradient in three species – *Betula pendula*, *Populus tremula* and *Tilia cordata* (from most shade intolerant to tolerant), and foliage photosynthetic potentials in *P. tremula* and *T. cordata*. Integrated quantum flux density (Q_i) incident on leaves was estimated with a method combining hemispherical photography and light measurements with quantum sensors made over the growing season. Long- and short-term light indices – average total seasonal daily integrated quantum flux density (T_s, mol m^{-2} d^{-1}) and that of the 3 d preceding foliage sampling (T_{3d}) – were calculated for each sampled leaf. In addition to total integrated quantum flux density, the part of Q_i attributable to direct flux was also computed. Strong linear relationships between the capacity for photosynthetic electron transport per area (J^a_{max}), estimated from *in situ* measurements of effective quantum yield of photosystem II (PS II), and Q_i averaged over the season and over the preceding 3 d were found for all studied species. However, the major determinant of J^a_{max}, the product of electron transport capacity per leaf dry mass (J^m_{max}) and leaf dry mass per area (M_A), was M_A rather than J^m_{max}, which was relatively constant along the light gradient. There was evidence that J^a_{max} is more tightly related to T_s, which characterizes the light climate during foliar development, than to short-term integrated light, possibly because there is little flexibility in adjustments in M_A after the completion of foliar growth. Leaf chlorophyll concentrations and the investment of leaf nitrogen in chlorophyll (Chl/N) were negatively related to Q_i – an investment pattern which improves light harvesting in low light. Xanthophyll cycle pool size (VAZ, violaxanthin + antheraxanthin + zeaxanthin) either expressed per unit chlorophyll (VAZ/Chl) or as a fraction of total carotenoids (VAZ/Car) increased with increasing Q_i in all species. However, contrary to J^a_{max}, it tended to correlate more strongly with short-term than with long-term average integrated light. There were few interspecific differences in J^a_{max}, Chl/N, VAZ/Chl and VAZ/Car when the variability in light level incident to the leaves was accounted for, indicating that the foliage of both shade-intolerant and -tolerant temperate tree species possesses considerable phenotypic flexibility. Collectively these results support the view that rapid adjustment of the xanthophyll cycle pool size provides an important means for acclimation to light fluctuations in a time scale of days, during which the potential for photosynthetic quenching of excitation energy is not likely to change appreciably.

Key-words: *Betula pendula*; *Populus tremula*; *Tilia cordata*; adaptation kinetics; carotenoids; photosynthetic electron transport; pigment composition; xanthophyll cycle.

INTRODUCTION

As a result of acclimation to growth light environment, foliar photosynthetic capacities per unit area consistently increase with increasing light reception at different locations in the canopy (Sims & Pearcy 1989; Ellsworth & Reich 1993; Pearcy & Sims 1994; Niinemets & Tenhunen 1997; Niinemets, Kull & Tenhunen 1998) – an adaptive response maximizing whole-canopy photosynthesis (Gutschick & Wiegel 1988; Pearcy & Sims 1994; Baldocchi & Harley 1995). Changes of foliar photosynthetic capacity along light gradients not only increase canopy carbon gain, but also imply an increasing potential to safely dissipate potentially damaging excitation energy via photosynthesis with increasing light level. However, given that the photosynthetic apparatus is expensive in terms of limiting resources such as nitrogen (e.g. Evans 1989), and that light changes dramatically on a diurnal basis, foliage photosynthetic capacities should not be adjusted to peak irradiances at midday, because such high and resource-demanding capacities would be wasted for most of the remaining day. Photosynthetic capacities

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should also not be tuned to maximum potential daily integrated quantum receipts which are infrequently observed on clear days, because realized annual average daily photon doses are less than the potential ones determined by site geographic latitude. In fact, there are indications that gas exchange of high light acclimated leaves is adjusted for efficiency under relatively low light conditions in natural stressful environments and with low nutrient availability (Tenhunen, Pearcy & Lange 1987). To cope with peak irradiances, a number of other less costly means for effective harmless quenching of excess excitation energy have evolved [cf. Demmig-Adams & Adams (1992b) for a review].

High light-triggered conversion of violaxanthin to zeaxanthin via antheraxanthin (termed the xanthophyll cycle) plays a central role in non-photochemical quenching of surplus excitation energy (Demmig-Adams & Adams 1992b, 1996; Pfündel & Bilger 1994). Although zeaxanthin, and possibly also antheraxanthin (Gilmore & Yamamoto 1993), is responsible for the quenching of excess excitation energy, the total pool of xanthophyll cycle carotenoids [violaxanthin + antheraxanthin + zeaxanthin (VAZ)] determines the capacity for zeaxanthin formation. Studies demonstrate that the VAZ pool per chlorophyll or total carotenoids consistently increases with increasing absorbed irradiance (Demmig-Adams *et al*. 1989, 1995; Thayer & Björkman 1990; Björkman & Demmig-Adams 1994; Bilger *et al*. 1995a; Königer *et al*. 1995; Logan *et al*. 1996). The VAZ pool size acclimates to a more than 20-fold step change in irradiance within 5–7 d (Demmig-Adams *et al*. 1989; Thayer & Björkman 1990; Björkman & Demmig-Adams 1994). This rapid adjustment in VAZ is relevant to understanding foliage acclimation to the strongly fluctuating light environment in natural canopies, where depending on unpredictable weather conditions such as cloudiness, the daily absorbed irradiance may vary by an order of magnitude from day to day. Because the adaptation of leaf photosynthetic capacity to high irradiance is predominantly morphological in tree species [i.e. mainly results from light-related adjustments in leaf dry mass per area, M_A (e.g. Kull & Niinemets 1993; Niinemets 1995, 1997a)] rather than from the changes in photosynthesis per dry mass [photosynthesis per area = photosynthesis per mass $\times M_A$ (e.g. Niinemets & Tenhunen 1997; Niinemets *et al*. 1998)], flexibility in photosynthetic acclimation to light fluctuations is restricted as soon as leaf thickness is fixed via lignification of cell walls. However, given the rapidity with which the VAZ pool size is altered, its dynamic tuning to frequent changes in the level of excess light may contribute strongly to photosynthetic adaptation to light environment.

To date, few studies have examined the relevant time scales of acclimation processes in woody plant canopies. Recently, the xanthophyll cycle was studied in three light environments within the tropical forest canopy (Königer *et al*. 1995), but due to a lack of reliable light measurements in the canopy, no quantitative relationships between VAZ pool size and canopy light environment were established. We studied the variability in leaf photosynthetic electron transport rate, pigment pool sizes and stoichiometry in three widely occurring European woody species of contrasting shade tolerance. *Betula pendula* Roth. and *Populus tremula* L. are shade-tolerant early-successional species, while *Tilia cordata* L. is a shade-tolerant late-successional forest component. The species have been ranked according to shade tolerance as *B. pendula* < *P. tremula* < *T. cordata* (Ellenberg 1988; Otto 1994).

The following hypotheses were tested: (1) foliage photosynthetic capacities are more strongly related to irradiance during leaf growth and development than to day to day light fluctuations; (2) VAZ pool size adjusts to the light gradient along the canopy as well as to the variability in light climate between the days. Because recent studies with genetically manipulated plants differing widely in photosynthetic capacity demonstrated that the xanthophyll cycle pool size is controlled by the level of excess excitation energy (Bilger *et al*. 1995a), we also suggested that (3) the VAZ pool size acclimates to excess rather than to total light reception. To characterize the strongly variable light environment specific to natural communities in more detail, several alternative light indices were used. Direct irradiance with high peak intensities is used as an estimate of excess light.

Often shade-adapted species sampled from low light locations exhibit a lower VAZ pool size than sun species from high light conditions (Thayer & Björkman 1990; Demmig-Adams & Adams 1992a; Johnson *et al*. 1993). Yet, in other experiments, both sun and shade species fit the same basic positive relationships between the potential for photochemical quenching of excitation energy and VAZ pool size (Thayer & Björkman 1990; Königer *et al*. 1995). Thus, the interspecific differences observed might largely be attributable to extreme differences in the light ranges sampled. Because current opinions regarding species effects on the xanthophyll cycle pool size are contradictory, we also asked here: (4) are there interspecific differences in the VAZ pool size at a common light exposure, and is the VAZ pool size dependent on species shade tolerance?

MATERIALS AND METHODS

Study sites

Betula pendula, *P. tremula* and *T. cordata* were investigated in Järvselja (58°22' N, 27°20' E, elevation 38–40 m), Estonia in July 1995. In this stand, the overstory (17–27 m) was dominated by *P. tremula* and *B. pendula*, *T. cordata* was the subcanopy species (4–17 m), and the understory was dominated by *Corylus avellana* L. and a coppice of *T. cordata*. The soil was a gleyed pseudopodsol formed on a loamy till with a C/N molar ratio of $23{\cdot}8 \pm 2{\cdot}2$, a pH_{KCl} of $4{\cdot}19 \pm 0{\cdot}10$, and a cation exchange capacity of $0{\cdot}61 \pm 0{\cdot}15$ mol kg^{-1} (base saturation $23 \pm 7\%$) in the humus horizon.

Another sample of *T. cordata* was taken during the first 2 weeks of August 1994 in a mixed stand near Tartu (58°15' N, 26°45' E, elevation 55–60 m above sea level),

Estonia. The overstory consisted of *T. cordata* and *Picea abies* (L.) Karst. There was no woody vegetation in the understory, but the herb cover, dominated by *Dactylis glomerata* L., *Phleum bertolonii* D.C. and *Poa pratensis* L., was dense and vigorous (height 0·7–1·0 m, coverage 100%). The soil – a brown pseudopodsol formed on a sandy clay moraine – had a thick (≈ 45 cm) humus horizon with a C/N molar ratio of 24 ± 9, a pH_{KCl} of 6·30 ± 0·15 and a cation exchange capacity of 2·3 ± 1·0 mol kg^{-1} (base saturation 94·9 ± 2·1%).

Foliage sampling

In all cases, the samples were taken between 1200 and 1400 h. In Järvselja, *B. pendula*, *P. tremula* and *T. cordata* were sampled on 12, 13, 19, and 28 July 1995. The canopy was accessed from permanent scaffolding (height 25 m) located at the study site, and the highest relative sampling height (sample height per total tree height) was always > 0·97. The mean [± standard error (SE)] height of the sampled trees was 24·8 ± 1·0 m in *B. pendula* (n = 3 trees), 25·0 ± 1·7 m in *P. tremula* (n = 4) and 15·1 ± 0·7 m in *T. cordata* (n = 4). For pigment analyses, discs of 1·03 cm^2 were removed from the leaves with a cork-borer, put in labelled vials and plunged into liquid nitrogen. In the laboratory, they were stored in air-tight sealed vials at – 18 °C until analysed. Separate control experiments demonstrated that leaf samples may be stored at this temperature for more than 1 year without any changes in pigment concentration and stoichiometry (W. Bilger, unpublished results). A subsample of five to nine leaves was taken from the same canopy locations for lamina dry mass per area and nitrogen determinations.

A mobile lift was used for foliage sampling in *T. cordata* at the Tartu site and the highest samples could be taken from the top of the trees (n = 4), which were 15 ± 2 m high. The sampling routine was the same as that in Järvselja, except that the leaves were initially put in plastic bags, held on ice, and the samples for pigment analyses were punched from the leaves in the laboratory (all within 1 h of collection).

Morphological and nitrogen analyses

Leaf circumference was traced with a computer digitizer (QD-1212, QTronix, Taiwan) and projected area calculated with a self-developed computer program. Petioles were discarded, and the leaflets were weighed after oven-drying at 70 °C for at least 48 h. Leaflet nitrogen concentrations were measured with an elemental analyser (CHN-O-Rapid, Foss Heraeus GmbH, Hanau, Germany).

Estimations of mean incident integrated quantum flux density (Q_i)

Continuous measurements of photosynthetically active quantum flux density (Q) with quantum sensors, and estimations of fractional penetration of irradiance at sensor locations with hemispherical photography were combined to derive long- and short-term average quantum flux densities incident on the leaves. In the Järvselja stand, Q was monitored at 18 canopy heights with eight GaAsP photodiodes (G1118, Hamamatsu Photonics K. K., Shizuoka, Japan) and 10 silicon photodiodes (OPT-21, Burr-Brown, Inc., Tucson, AZ, USA). All photodiodes were equipped with Teflon diffusers to improve the cosine response, and silicon photodiodes were equipped with short-pass detector trimmers (400–690 nm, Optical Coating Laboratory, Inc., Santa Rosa, CA, USA) to remove the infrared spectral bands. Both types of photodiodes were calibrated against a quantum sensor (LI-190SA, Li-Cor, Inc., Lincoln, NE, USA). Daily integrated Q (Q_i) was calculated from the sensor readings taken in 1 min steps. The mean seasonal total integrated quantum flux density (T_s, mol m^{-2} d^{-1}) for the sensor position was found as the average daily integrated Q between the completion of lamina expansion growth (approximately 3 June 1995) and the date of foliage sampling. Hemispheric photographs were taken just above the sensor locations at weekly intervals. The fractions of penetrating diffuse (I_{dif}, diffuse site factor) and of potential penetrating direct solar radiation of open sky (I_{dir}, direct site factor) were calculated from these photographs as described previously (Niinemets & Kull 1998). Hemispherical photographs were also taken from the sample locations immediately after foliage collection. Q_is for the sample locations were calculated from multiple linear regression equations in the form of $T_s = a \times I_{dif} + b \times I_{dir}$. The regression coefficients, a and b, were estimated from actual T_s measurements with the quantum sensors. Because the intercept was generally insignificantly different from zero ($P > 0{\cdot}05$), and it was also reasonable to assume that it is truly zero, statistical models did not include an intercept term. Thus, all deviations in the regression formulas were taken with respect to zero [see Sokal & Rohlf (1995)], resulting in statistical equations with high predictive capability (r^2 averaging 0·99, $P < 0{\cdot}001$). Nevertheless, r^2 was always larger than 0·96 ($P < 0{\cdot}001$) even in the statistical models forced to include an intercept.

Average integrated Q for the 3 days preceding foliage sampling and fluorescence measurements (T_{3d}) was computed in an analogous manner. Although the acclimation of the xanthophyll cycle to a step change in incident light conditions may take ≈ 5 d (cf. Introduction), we used the daily integrated Q averaged over the 3 d preceding sample collection for 'short-term' Q_i, because this was the longest uniform light period during sampling. Average integrated values for direct irradiance were calculated as the products of b and I_{dir} (D_s for average seasonal and D_{3d} for the direct light averaged over the 3 d preceding the measurements). b estimations from daily time-courses of Q were in close agreement with those obtained from regression analyses (data not shown).

No direct measurements of Q were available for *T. cordata* in the Tartu stand. However, because the ratio of global solar radiation to Q_i is very conservative over the long term (e.g. Meek *et al*. 1984), a correlation between global solar radiation and Q_i was employed to calculate Q_i.

First, 'global site factor', the fractional penetration of solar radiation in the photosynthetically active spectral region, I_{sum}, was computed as:

$$I_{sum} = p_{dif}I_{dif} + (1 - p_{dif})I_{dir}, \quad (1)$$

where p_{dif} is the ratio of diffuse irradiance to total irradiance in the photosynthetically active spectral region above the canopy. p_{dif} depends on long-term cloudiness conditions as well as on the differences in light spectral quality between diffuse and direct irradiance, and was equal to 0·574 for the Tartu stand (average from June to August 1994; Tôravere Meteorological Station, 58°16' N, 26°28' E, unpublished results). The ratio of Q_i to the mean seasonal global radiation of 1·92 mol MJ^{-1} was obtained from the measurements at Järvselja and Tôravere in 1995 (r^2 = 0·86, P < 0·001 for a seasonal relationship between daily global solar radiation and Q_i). This value was used to calculate average Q_i above the canopy (Q^0_i; I_{sum} = 1·0) during the season (37·7 mol m^{-2} d^{-1} for 1 June 1994 to 15 August 1994) and during the 3 d preceding the foliage sampling. Q_i for different sample locations is the product of I_{sum} and Q^0_i. Q_i attributable to direct flux was calculated from the values of global direct solar radiation after correcting for spectral quality effects. Thus, in all instances four descriptors of light environment incident on leaves could be calculated: seasonal average total (T_s) and direct (D_s) daily integrated Q; and the total (T_{3d}) and direct (D_{3d}) daily integrated Q averaged over the 3 d preceding foliar sampling.

Pigment analysis

The samples were ground in dim light in liquid nitrogen in the presence of quartz sand and $MgCO_3$, extracted on ice with high performance liquid chromatography (HPLC) grade 100% acetone (Carl Roth GmbH, Karlsruhe, Germany), and centrifuged at 0 °C and 5000 *g* for 3 min. The pellet was further extracted with a small amount of acetone until the supernatant remained colourless, but the re-extraction was repeated at least twice. Water was added to the combined supernatants to give a final concentration of acetone of 80% (v/v). The pigment solution was filtered through a 0·45 μm syringe filter before injection into HPLC.

Carotenoid composition was analysed with reversed-phase HPLC according to a modified method of Büch *et al*. (1994) using a Hypersil ODS column (particle size 5 μm, column length 250 mm, inner diameter 4·6 mm; Alltech Associates Inc., Deerfield, IL, USA) which was thermostated at 10 ± 0·1 °C (Gynkotek GmbH, Munich, Germany). The system, with two mixing pumps (Model 510, Waters Millipore, Milford, USA), was the same as described by Bilger *et al*. (1995a). The pigments were eluted at a flow rate of 1·5 cm^3 min^{-1}. The mixture of 25% solvent A (H_2O, Hepes 0·1 M, pH 8·0) and 75% solvent B (100% acetone) was run isocratically for the first 7·5 min, followed by a 9·5 min linear gradient to 100% B, which was run isocratically for 3 min. The eluent composition was further changed to 25% A and 75% B by a 2 min linear gradient, and the column was equilibrated for 8 min before the next sample was injected.

The HPLC was calibrated using purified or commercially available pigment standards. Violaxanthin, neoxanthin and lutein were prepared by thin-layer chromatography as described in Demmig *et al*. (1987), zeaxanthin was purchased from Carl Roth, and β-carotene was purchased from Sigma-Aldrich GmbH (Deisenhofen, Germany). A pigment extract in 80% aqueous acetone, obtained from fresh leaves of *Spinacia oleracea* L. as described above, was used for calibration of chlorophylls *a* and *b*. The pigment concentrations of the calibration solutions were calculated from extinction measurements (Uvikon Spectrophotometer, Model 930, Milan, Italy) using appropriate wavelengths and extinction coefficients for carotenoids (Davies 1976) and for chlorophylls (Porra, Thompson & Kriedemann 1989). The calibration factor for violaxanthin was also used for antheraxanthin. In addition to major pigment peaks, a number of minor carotenoids such as α- and β-cryptoxanthin, lutein-5,6-epoxide and α-carotene was regularly detected in chromatograms. For the calculation of total foliar carotenoid content, peak areas of minor carotenoids were converted to concentrations using the calibration factors obtained for structurally similar major carotenoids. Nevertheless, because the content of minor carotenoids estimated in this way was < 3% of the total carotenoids, it is unlikely that these simplifications resulted in an appreciable error in total carotenoid calculations.

Chlorophyll fluorescence measurements

The steady-state fluorescence yield (F_s) and the fluorescence yield after the application of a saturating pulse of white light (F_m') were measured with a portable pulse-modulation fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) equipped with a leaf clip holder [model 2030-B, see Bilger, Schreiber & Bock (1995b) for a thorough description] *in situ* in leaves of *P. tremula* and *T. cordata* along the canopy light gradient on 19 July 1995 between 1300 and 1600 h. Saturating pulse kinetics were checked before each measurement series at a given canopy height, and the intensity and length of the saturated pulse were adjusted to fully close all photosystem II (PS II) reaction centres, but to avoid photoinhibitory damage of the samples. In general, the required pulse lengths and intensities were greater for overstory samples. Natural light was used during the F_s measurements on 19 July, and the steady-state values were sampled after the leaves had been exposed to direct beam irradiance for at least 10 min. In the same leaf, F_s and F_m' were measured at three to four locations along the mid-rib. Care was taken to ensure that both the micro-quantum sensor of the leaf clip (type BPX 91B, Siemens AG, Germany) and the sampled leaf area were in direct beam light during measurements. The rate of photosynthetic electron transport (μmol e^- m^{-2} s^{-1}) was calculated according to Genty, Briantais & Baker (1989):

$$J = 0{\cdot}5\phi_{II}\xi Q, \quad (2)$$

where Q is the incident quantum flux density (μmol m^{-2} s^{-1}), ξ is leaf absorptance, and ϕ_{II}, the effective quantum yield of PS II, was computed as $(F_m' - F_s)/F_m'$. J measured at quantum flux densities assumed to be saturating for the electron transport (1100–1700 μmol m^{-2} s^{-1} for the upper canopy leaves and 700–900 μmol m^{-2} s^{-1} for the lower canopy leaves) was used as an estimate for the capacity of photosynthetic electron transport (J_{max}). ξ was calculated from the leaf chlorophyll content per area with an empirical equation found for a large number of species and chlorophyll contents (cf. Niinemets & Tenhunen 1997). Implicit in Eqn 2 is that both photosystems, PS I and PS II, intercept equal amounts of light. Because the ratio of quantum yields of PS II and photosynthetic O_2 evolution (Seaton & Walker 1990; Öquist & Chow 1992) or CO_2 absorption (Oberhuber, Dai & Edwards 1993; Valentini *et al*. 1995) at high light and saturating CO_2 concentrations is very conservative, chlorophyll fluorescence analysis may safely be used as a reliable tool for rapid assessments of foliar electron transport potentials (Schreiber, Bilger & Neubauer 1994).

To check whether the natural light intensities used for J_{max} determinations were saturating, two additional series of measurements with artificial illumination were conducted with detached twigs of *P. tremula* on 21 July, and with attached leaves of *P. tremula* and *T. cordata* on 28 July 1995. On 21 July, shoots with three to four leaves were cut under water, thrown down, immediately recut under water, and fluorescence parameters measured within 1 h of collection with PAM-2000. Starting at darkness, Q was increased in steps, allowing the fluorescence parameters to reach steady state. Q, provided by a halogen lamp (Decostar 51-S, Osram, Berlin, Germany), was varied by adjusting the distance between the sample and the light source (10–30 cm), whereas the micro-quantum sensor was always held in the beam of the artificial light. The halogen light was filtered through a layer of water (2 cm) to reduce the contribution of infrared wavelengths.

Illumination provided by an internal halogen lamp of the PAM-2000 (Bellaphot, Osram GmbH, Munich, Germany) equipped with a short-pass filter (Calflex-X special, Balzers AG, Liechtenstein) was used during the *in situ* measurements on 28 July. The distance between the centre of the fibreoptics' endpiece and the sample was fixed at 6·7 mm. The centre of the micro-quantum sensor was held at 6·3 mm relative to the outer boundary of the sample [see Bilger *et al.* (1995b)]. This distance ensured that the sensor was in the light beam, and that the shading of the sample by the sensor was low. It was necessary to construct an empirical model for the calculation of Q at the leaf surface during the measurements with the internal halogen light, because: (1) the distance between the light source and the sample was small in this case; (2) the quantum sensor of the leaf clip is located 2·35 mm above the plane of the leaf surface; and (3) the sensor could not be held in the leaf region where the illumination was the brightest because of the requirements for minimal leaf shading by the sensor. The light measurements with various sensor locations and heights with respect to the fibreoptics' exit-plane demonstrated that with the distances of the sensor and fibreoptics relative to the sample used in the current study, Q at the leaf surface was overestimated by the quantum sensor by a factor of 1·29. Thus, all Q values were corrected by this empirical factor before J (Eqn 2) was calculated. No corrections were necessary for the measurements with natural light and the external halogen lamp because the distance between the light source and the sample was much greater than the distance between the sensor and the leaf surface.

On both 21 and 28 July, the experiment was continued until the on-line calculated electron transport rates did not increase with additional increases in Q (Fig. 1). Depending on the leaf location in the canopy, this occurred at 1000–3000 μmol m^{-2} s^{-1}, suggesting that J_{max} may have been underestimated in several *in situ* estimations accomplished with natural light. However, leaf temperature, measured with a thermocouple attached to the abaxial side of the leaf, was not controlled during the fluorescence measurements, and varied depending on air temperature and Q from 20·9 to 30·3 °C for natural and from 18·0 to 33·5 °C for artificial Q used in J_{max} calculations. Therefore, before J_{max} values obtained for different leaf regions were averaged, all J_{max} estimates were converted to 25 °C using an empirical relationship between the capacity for uncoupled chloroplastic electron transport and temperature measured in *Hordeum vulgare* [Nolan & Smillie (1976), temperature constants in Niinemets & Tenhunen (1997)]. When the positive correlation between the measurement Q and the

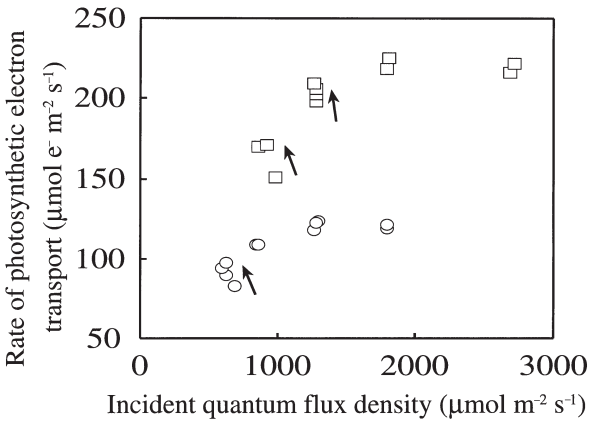

Figure 1. Examples of the measurement of the capacity for photosynthetic electron transport (J_{max}) with a pulse-amplitude modulated fluorometer (PAM-2000) using artificial light provided by an external halogen lamp (Bellaphot, Osram GmbH, Germany). At each light level, the effective quantum yield of photosystem II of attached leaves was monitored until steady-state values were reached. The arrows indicate changes in the rate of electron transport with time after changing the light level. All measured values of electron transport were standardized to a common temperature as described in Materials and Methods, and J_{max} was defined as the highest observed estimate per curve. ○, a leaf of *Tilia cordata* grown at an average seasonal daily quantum flux density (T_s) of 3·6 mol m^{-2} d^{-1}; □, *Populus tremula*, T_s = 14·6 mol m^{-2} d^{-1}.

leaf temperature was accounted for, J_{max} measured with artificial light appeared to saturate at Qs comparable to those used in *in situ* estimations.

RESULTS

Canopy light environment

Differences in daily photon input were more than 20-fold between the upper and lower canopy leaves on clear (Fig. 2a) as well as on overcast (data not shown) days, resulting in a similar range of variation for direct and total light estimates across the canopy when daily integrated quantum flux density was averaged over the season (Fig. 2b). All four light descriptors – seasonal average total and direct, and 3 d average total and direct daily integrated quantum flux density – were correlated with each other for the entire set of values (Fig. 2c & d), indicating that the relative light rankings of various canopy positions were remarkably conservative. Nevertheless, depending on sample location in the canopy, integrated direct quantum flux densities varied more than three-fold at a common total average flux density (Fig. 2b–d). By the same token, 3 d average light values varied by a factor of three at a common average seasonal Q_i between the overcast (during sampling of *T. cordata* in the Tartu stand) and bright days (during the sampling in Järvselja).

In spite of the large variability observed, the strong autocorrelations between various light descriptors in the natural

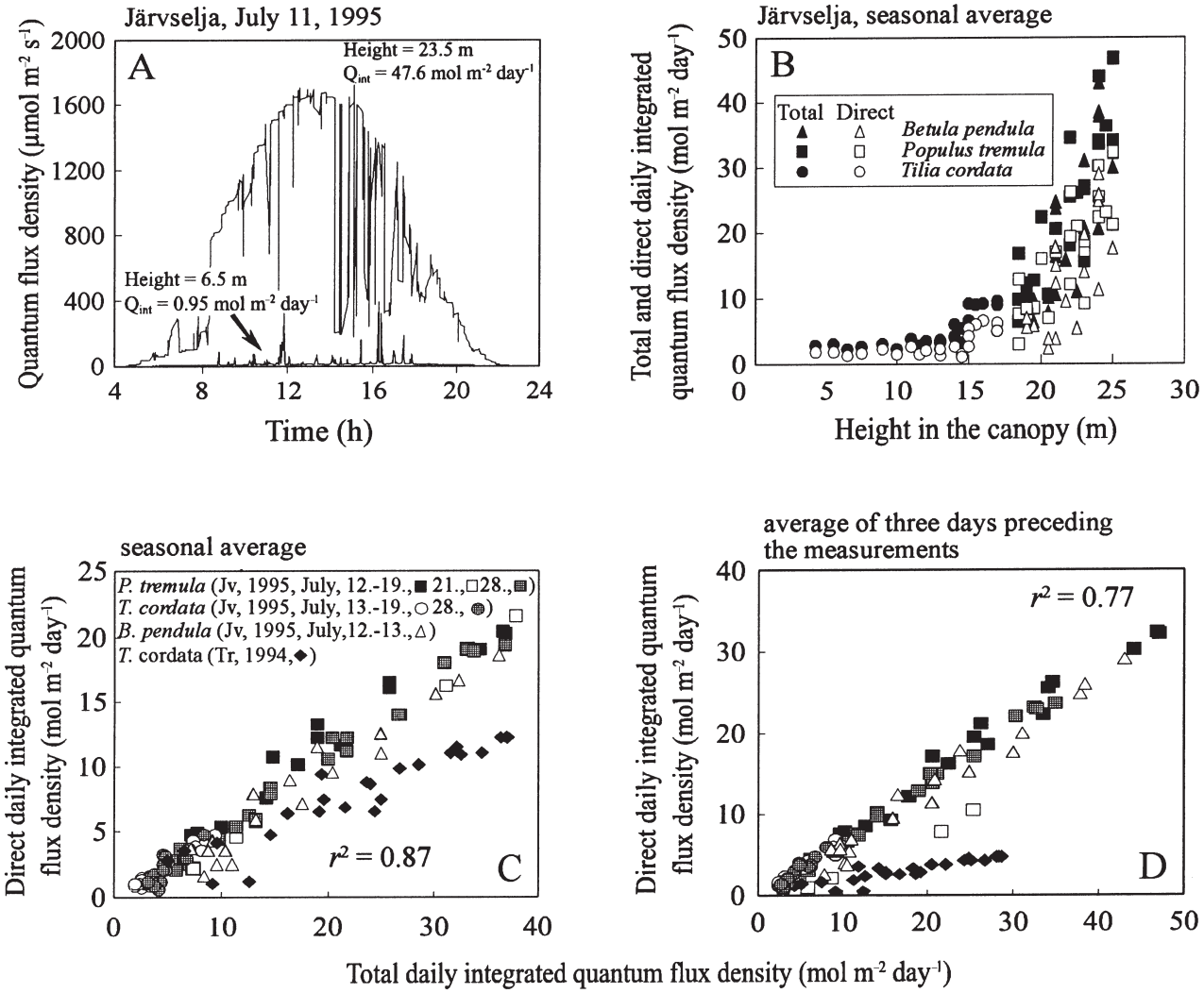

Figure 2. Diurnal variation in light environment in the upper and lower canopy on a bright day (a), and summary of the total (closed symbols) and direct (open symbols) seasonal (from the completion of the expansion growth of leaf area until foliage sampling) average daily integrated quantum flux densities (Q) at the sampling locations (b) in the deciduous mixed stand at Järvselja (58°22' N, 27°20' E). Correlations between total (T_s) and direct (D_s) daily integrated Q averaged over the whole season (c); and total (T_{3d}) and direct (D_{3d}) daily integrated Q averaged over the 3 d preceding foliar sampling (d). r^2s are calculated for all species and all sample points. Jv is the abbreviation for the Järvselja stand and Tr for the Tartu stand (58°15' N, 26°45' E). Other correlations are 0·934 (T_s versus T_{3d}), 0·951 (D_s versus T_{3d}), 0·682 (D_{3d} versus T_s), and 0·861 (D_s versus D_{3d}). All correlations with light descriptors were significant at $P < 0·001$ ($n = 134$).

canopy made it difficult to separate the physiological effects of total irradiances from those of direct ones, and short- and long-term light quantity effects. In general, when a correlation was detected with one light variable, the relationship was qualitatively similar with the other three; even though the correlation coefficients were substantially different with various light estimates in some cases, the differences were not clear-cut in other comparisons. Therefore, for a better separation, an alternative routine was also employed: (1) the linear regression was calculated between the two light variables, the effects of which on photosynthetic electron transport or on pigment pools were compared (e.g. Fig. 2c, d); (2) for each y value used in the regression, a studentized residual was computed (Velleman & Welsch 1981); (3) only the data points with y residuals of this regression ≥ 1·0 were included in the comparison of the influence of the two light estimates on foliar physiology and chemistry. Thus, the data points with the light descriptors which were the least correlated with each other, and deviated the most from the autocorrelation line were selected by this routine. The studentized residual follows a t-distribution, and the probability of having values with residuals ≥ 1·0 by chance is ≤ 0·3 for the degrees of freedom available in the current study (Sokal & Rohlf 1995).

Acclimation of photosynthetic electron transport to incident light

A strong positive correlation between the maximum values of photosynthetic electron transport rate per area (J^a_{max}) and average seasonal Q_i (T_s) was observed for both *P. tremula* and *T. cordata* (Fig. 3a). Although the differences in the determination coefficients of the linear regressions were small, the relationships tended to be weaker with the integrated light values of the three preceding days (T_{3d}; r^2 = 0·65 for *P. tremula* and r^2 = 0·66 for *T. cordata*), suggesting that J^a_{max} was adjusted to long-term rather than to short-term integrated light values. To gain more conclusive evidence for this suggestion, a sample consisting of points with light values deviating the most from the T_s versus T_{3d} regression line was constructed in *P. tremula* (n = 8; in *T. cordata*, no sample points satisfied the criterion of studentized residual ≥ 1·0). In this new sample, T_s versus T_{3d} were not correlated (r^2 = 0·38, P > 0·1), yet the correlation of J^a_{max} with T_s was highly significant (r^2 = 0·81, P < 0·005), but insignificant with T_{3d} (r^2 = 0·15, P > 0·4).

The slopes of J^a_{max} versus T_s (Fig. 3a) and J_{max} per unit dry mass (J^m_{max}) versus T_s (Fig. 3b) were greater in *T. cordata* than in *P. tremula* (P < 0·001 according to a separate slope ANCOVA). This was attributable to an increasing investment of leaf nitrogen in electron transport machinery with increasing T_s in *T. cordata* (Fig. 3c). Because the nitrogen concentration was greater in leaves of *T. cordata* (1·988 ± 0·027 mmol g^{-1}) than in *P. tremula* (1·596 ± 0·019 mmol g^{-1}, means are different at P < 0·001 according to one-way ANOVA), J^m_{max} was larger in leaves of *T. cordata* exposed to the highest growth light levels observed than in low light leaves of *P. tremula* (Fig. 3b).

The primary determinant of the high positive correlations between J^a_{max} and the light descriptors was a strong positive correlation between leaf dry mass per area (M_A) and seasonal average Q_i (Fig. 4a). The electron transport rates expressed per unit leaf dry mass were less strongly related to long-term light environment (Fig. 3b) than the values expressed per unit surface area (rate per area = rate per mass × M_A).

Changes in total chlorophyll and carotenoids in response to canopy light environment

Total chlorophyll (a + b) content per area was generally weakly (r^2s within species were around 0·30, P < 0·001) but positively related to seasonal Q_i in all species. This slight increase resulted from the increase in M_A with increasing seasonal average Q_i (Fig. 4a), as chlorophyll per dry mass actually decreased with increasing integrated light (Fig. 4b), reflecting a greater foliar nitrogen investment in light harvesting at low light (Fig. 4c). In

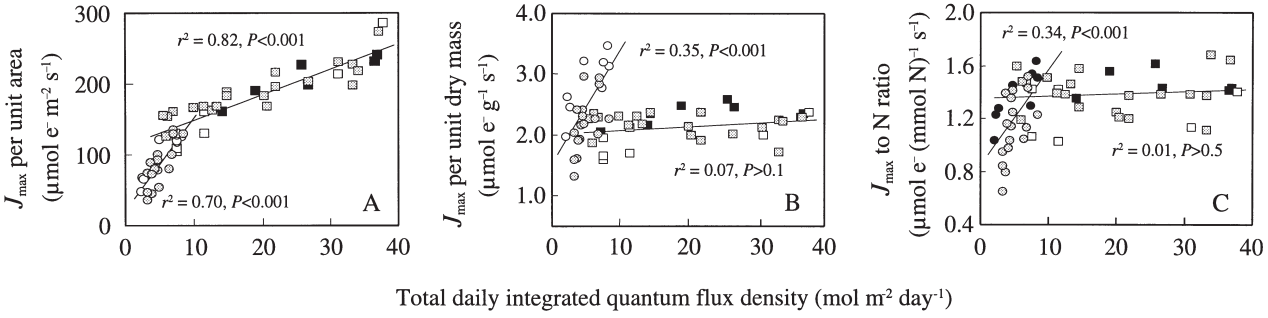

Figure 3. Dependence of the capacity for photosynthetic electron transport (J_{max}) per unit leaf area (a) and per unit leaf dry mass (b**)** and the ratio of J_{max} to leaf N (c) on seasonal average total daily integrated quantum flux density (T_s). J_{max} was calculated from chlorophyll fluorescence analysis (Eqn 2), and was standardized to 25 °C as described in Materials and Methods. Symbols as in Fig. 2c, but open symbols (□, *P. tremula*; ○, *T. cordata*) denote measurements with natural illumination, and filled and dotted symbols (■, ▨, *P. tremula*; ●, *T. cordata*) with artificial illumination.

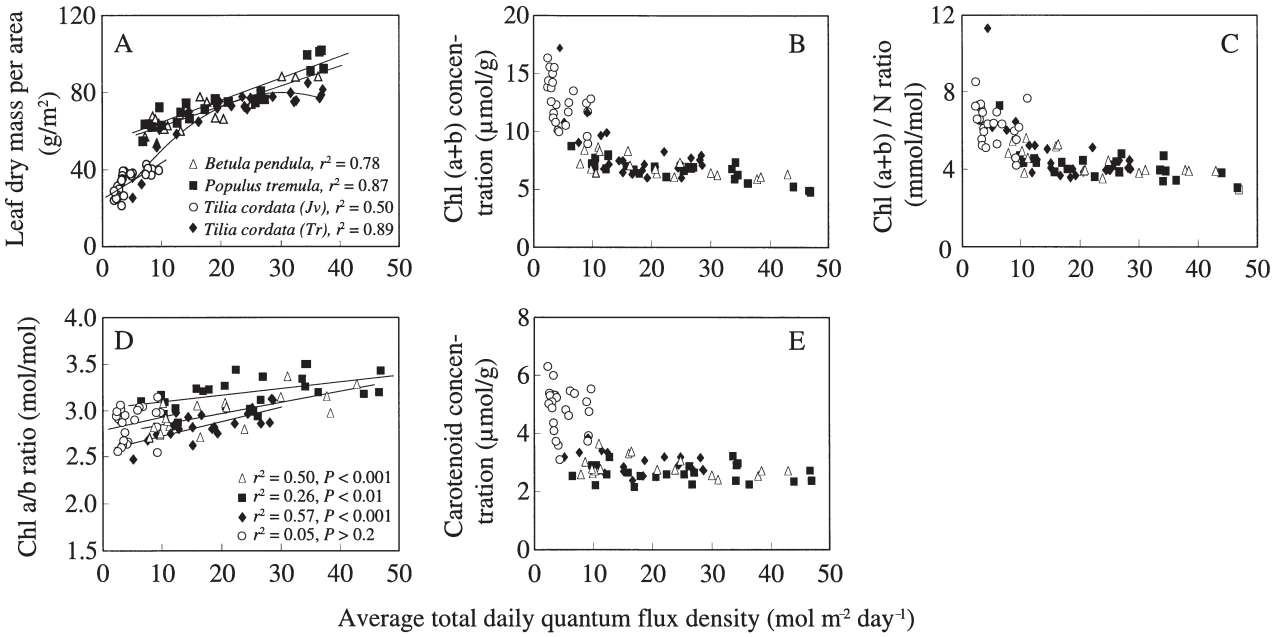

Figure 4. Acclimation of leaf dry mass per area (M_A, a), concentration of foliar chlorophylls (b), chlorophyll to nitrogen molar ratio (c), chlorophyll *a*/*b* ratio (d), and concentration of total carotenoids (e) to light environment. M_A is given in relation to seasonal daily integrated quantum flux density (T_s), and pigment pools and ratios in relation to daily integrated quantum flux density averaged over the 3 d preceding foliar sampling (T_{3d}). *Jv* indicates samples collected from Järvselja and *Tr* from the Tartu stand. r^2s are for second-order polynomial [*T. cordata* from Tartu stand in (a)] and linear (all others) regressions. $P < 0{\cdot}001$ for all regressions depicted in (a).

contrast to the relationships with J_{max}, the hyperbolic dependencies between Chl/mass and Chl/N tended to be less scattered with Q_i integrated over the 3 d preceding foliar sampling than with the seasonal light descriptors. Even though T_{3d} and T_s were weakly correlated in the data set including the cases with light values deviating the most from the T_{3d} versus T_s regression line (n = 32, $r^2 = 0{\cdot}39$, $P < 0{\cdot}001$), Chl/mass and Chl/N were negatively correlated with T_{3d} but not with T_s (Table 1) in this data set. Because M_A was more strongly related to seasonal than to short-term light climate, T_s was a better correlate with Chl/area ($r^2 = 0{\cdot}42$, $P < 0{\cdot}001$ for all cases) than was T_{3d} ($r^2 = 0{\cdot}22$, $P < 0{\cdot}001$ for all cases, see also Table 1). As with total chlorophyll, total carotenoid content per leaf dry mass was constant over most of the light range (Fig. 4e), but due to a greater M_A at high light, there was a moderate increase in carotenoid content per leaf area in all cases (r^2s with T_s varied from 0·37 to 0·50 within species).

For all of the material, leaf chlorophyll and total carotenoid concentrations scaled positively with leaf N concentration ($r^2 = 0{\cdot}42$ for chlorophyll, and $r^2 = 0{\cdot}52$ for

Dependent variable	Independent variable	Intercept[2]	Slope	r^2	P
Chl/mass (μmol g^{-1})	T_{3d}	8·09	−0·051	0·35	0·001
Chl/mass (μmol g^{-1})	T_s	7·19	−0·019	0·02	0·40
Chl/area (μmol m^{-2})	T_{3d}	592	−1·52	0·04	0·26
Chl/area (μmol m^{-2})	T_s	427	4·48	0·18	0·02
Chl/N (mmol mol^{-1})	T_{3d}	4·57	−0·020	0·15	0·03
Chl/N (mmol mol^{-1})	T_s	4·53	−0·018	0·06	0·16
Chl *a*/*b* (mol mol^{-1})	T_{3d}	2·70	0·014	0·39	0·001
Chl *a*/*b* (mol mol^{-1})	T_s	3·03	0·002	0·00	0·77

Table 1 Effects of total integrated daily quantum flux density averaged over the season (T_s, mol m^{-2} d^{-1}) and over the 3 d preceding foliar sampling (T_{3d}) on foliage pigment content and stoichiometry: results of simple linear regression analyses (n = 32, all species pooled)[1]

[1]To reduce the influence of autocorrelation between various light descriptors on the comparisons, the data points used in the regressions were extracted from the whole set of data by first calculating the T_{3d} versus T_s regression line, and thereafter sorting out the cases with light values that deviated the most from this regression (studentized residual ≥ 1·0, see, for example Fig. 2c,d for the strong autocorrelations between light estimates). [2]All intercepts were significantly different from zero at $P < 0{\cdot}001$.

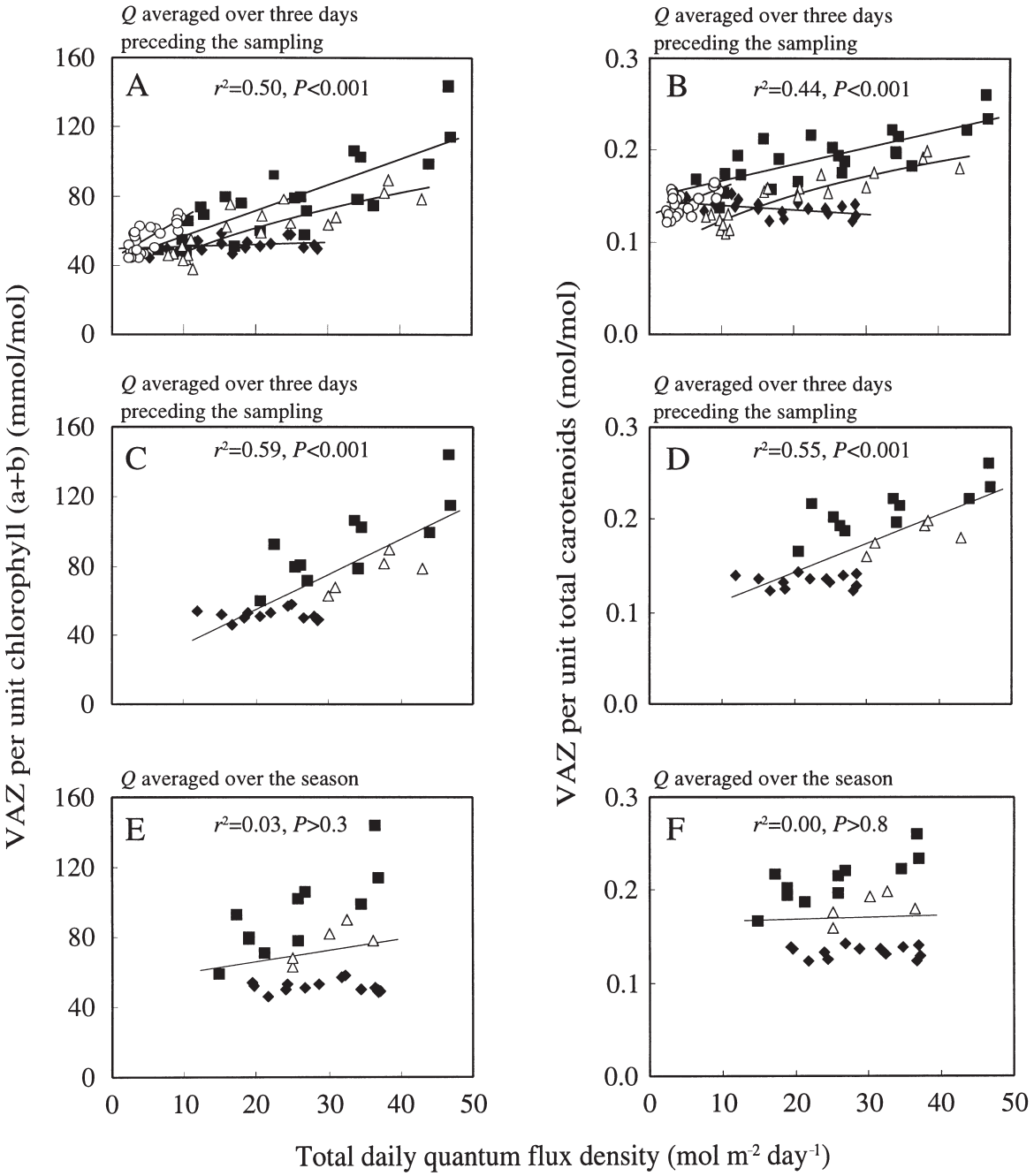

Figure 5. Relationships between VAZ (sum of violaxanthin, antheraxanthin and zeaxanthin) per chlorophyll and total carotenoids, and total daily quantum flux density averaged over the 3 d preceding foliar sampling (T_{3d}; a–d) and over the season (T_s; e, f). Because the integrated quantum flux densities averaged over the season and over the 3 d preceding the sampling were correlated (cf. the legend of Fig. 2), only the points from (a) and (b) with light values that deviated the most from the regression line of T_s versus T_{3d} (studentized residual ≥ 1·0, see also Fig. 2c,d) were included in the comparison of the effect of different light estimates on VAZ in (c)–(f). All correlations depicted in the figures are for the whole set of values. The coefficients of determination for the regressions within species in (a) and (b) are: *Betula pendula* (△), $r^2 = 0{\cdot}72$, $P < 0{\cdot}001$ for VAZ/Chl and $r^2 = 0{\cdot}82$, $P < 0{\cdot}001$ for VAZ/Car; *Populus tremula* (■), $r^2 = 0{\cdot}63$, $P < 0{\cdot}001$ for VAZ/Chl and $r^2 = 0{\cdot}58$, $P < 0{\cdot}001$ for VAZ/Car; *Tilia cordata* (Järvselja, ○), $r^2 = 0{\cdot}54$, $P < 0{\cdot}001$ for VAZ/Chl and $r^2 = 0{\cdot}36$, $P < 0{\cdot}005$ for VAZ/Car; *T. cordata* (Tartu, ◆), $r^2 = 0{\cdot}09$, $P > 0{\cdot}2$ for VAZ/Chl and $r^2 = 0{\cdot}24$, $P < 0{\cdot}05$ for VAZ/Car.

carotenoids, $P < 0{\cdot}001$ for both), and the variability in leaf chlorophyll (Fig. 4b) and carotenoids (data not shown) per N was considerably less than that in the pigment pools expressed per leaf dry mass.

Changes in pigment stoichiometry along canopy light gradients over the short and long term

The chlorophyll *a*/*b* ratio was positively correlated with T_s

and T_{3d} in all cases, except for *T. cordata* in Järvselja (Fig. 4d). T_{3d} described a slightly larger fraction of the total variance in the chlorophyll *a*/*b* ratio (r^2 = 0·39, P < 0·001 for all cases) than T_s (r^2 = 0·23, P < 0·001, see also Table 1).

The xanthophyll cycle pool size (VAZ) either expressed on total leaf chlorophyll (Fig. 5a,c,e) or as a fraction of leaf carotenoids (Fig. 5b,d,f) was tightly related to Q_i. For the whole material, the correlations were more significant with 3 d average light (Fig. 5a,b) than with T_s (r^2 = 0·24 for VAZ/Chl and r^2 = 0·19 for VAZ/Car for all cases pooled), and VAZ appeared to be independent of T_s in a sample where the autocorrelation between the two light descriptors was low (Fig. 5c–f). Similar relationships were also found when VAZ was expressed per unit lamina area or dry mass (data not shown). Although all species exhibited qualitatively identical patterns in VAZ versus T_s relationships, the more shade-tolerant species *T. cordata* (Järvselja stand) and *P. tremula* had higher intercepts of VAZ/Chl versus T_{3d} (Fig. 5a), and VAZ/Car versus T_{3d} (Fig. 5b) than *B. pendula* (P < 0·001 for all comparisons according to a common slope ANCOVA).

As a result of the covariation of VAZ (Fig. 5) and J^a_{max} (Fig. 3a) with Q_i, VAZ, characterizing the capacity for non-photochemical excitation energy quenching, and J_{max}, that for photochemical quenching, were positively correlated (Fig. 6).

When all species were pooled, direct light integrated over the 3 d preceding foliar sampling (D_{3d}), described a larger fraction of total variance than T_{3d} in the relationships with VAZ (cf. Figs 5a,b & 7a,b). There was also an important distinction between D_{3d} and T_{3d} in the data sets of *T. cordata*. VAZ/Chl and VAZ/Car were positively correlated with T_{3d} in *T. cordata* sampled on bright days in Järvselja, but not in *T. cordata* collected on overcast days in Tartu, and the correlation within the pooled set of values was insignificant (Fig. 7c). Yet, both sets of data fit the same relationship with D_{3d} (Fig. 7d), suggesting that the xanthophyll cycle pool size was adjusted to direct rather than to total irradiances.

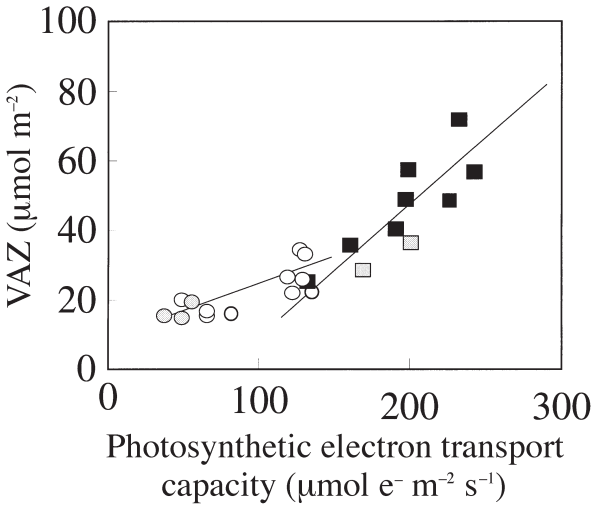

Figure 6. Correlation between VAZ (sum of violaxanthin, antheraxanthin and zeaxanthin) and photosynthetic electron transport capacity per area. Symbols as in Fig. 3c. r^2 = 0·73 (P < 0·001) for both *Tilia cordata* and *Populus tremula*.

DISCUSSION

Acclimation of foliar photosynthetic capacities to canopy light gradients

We observed that there was a strong relationship between J^a_{max} and Q_i (Fig. 3a). Thus, the studied species adjusted their potentials for photosynthetic electron transport to incident light – a response compatible with previously detected patterns among a wide range of species (cf. Introduction). Moreover, the dependence of J^a_{max} on canopy light environment should also imply that foliage photosynthetic rates measured at saturating irradiance and ambient CO_2 concentrations increase with increasing integrated average light receipt, because there is a basic positive dependence between the maximum activity of ribulose bisphosphate-1,5-carboxylase/oxygenase (Rubisco; V_{cmax}) and J_{max} (Wullschleger 1993; Leuning 1997).

In herbaceous species, the adjustments in thylakoid stoichiometry with respect to increasing Q_i, enhancing the capacity for photosynthetic electron transport, may proceed fairly rapidly [within 5–7 d, see, for example Grahl & Wild (1975), Chow & Anderson (1987a,b)]. However, such rapid changes have not been observed in tree species where the leaves are relatively long-lived with heavily lignified cell walls, and where leaf anatomy is unresponsive to environmental conditions after leaf development has been completed. Although the various light descriptors were correlated, J^a_{max} was more strongly related to seasonal average Q_i than to short-term light fluctuations in the current study (see also the data analysis after the autocorrelation between the light estimates has been removed). We suggest that the stronger correlation of J^a_{max} with T_s than with T_{3d} results from the circumstance that J^a_{max} was mostly determined by foliar anatomy (M_A, Fig. 4a; cf. Fig. 3a,b). The alterations in the capacity for electron transport per unit dry mass played only a relatively minor role: across the whole set of values, J^a_{max} varied by eight-fold (Fig. 3a), but the J_{max} to N ratio varied by 2·5-fold (Fig. 3c).

The observed differences in J^a_{max} and J^m_{max} between *P. tremula* and *T. cordata* are interesting (Fig. 3a,b). Generally, shade-tolerant species have lower nitrogen concentrations (Küppers 1994; Niinemets 1997b) and lower nitrogen investments in the proteins of the photosynthetic electron transport chain (Niinemets & Tenhunen 1997; Niinemets *et al.* 1998). In a previous study, *P. tremula* had indeed greater nitrogen investments in thylakoid proteins limiting J_{max} (Niinemets *et al.* 1998). By contrast, *T. cordata* coexisting with *P. tremula* in the same stand had greater foliar nitrogen concentrations in the current study. A literature review for *Acer saccharum* revealed that the nitrogen investment in photosynthetic electron transport is constant over most of the light range, and decreases in low

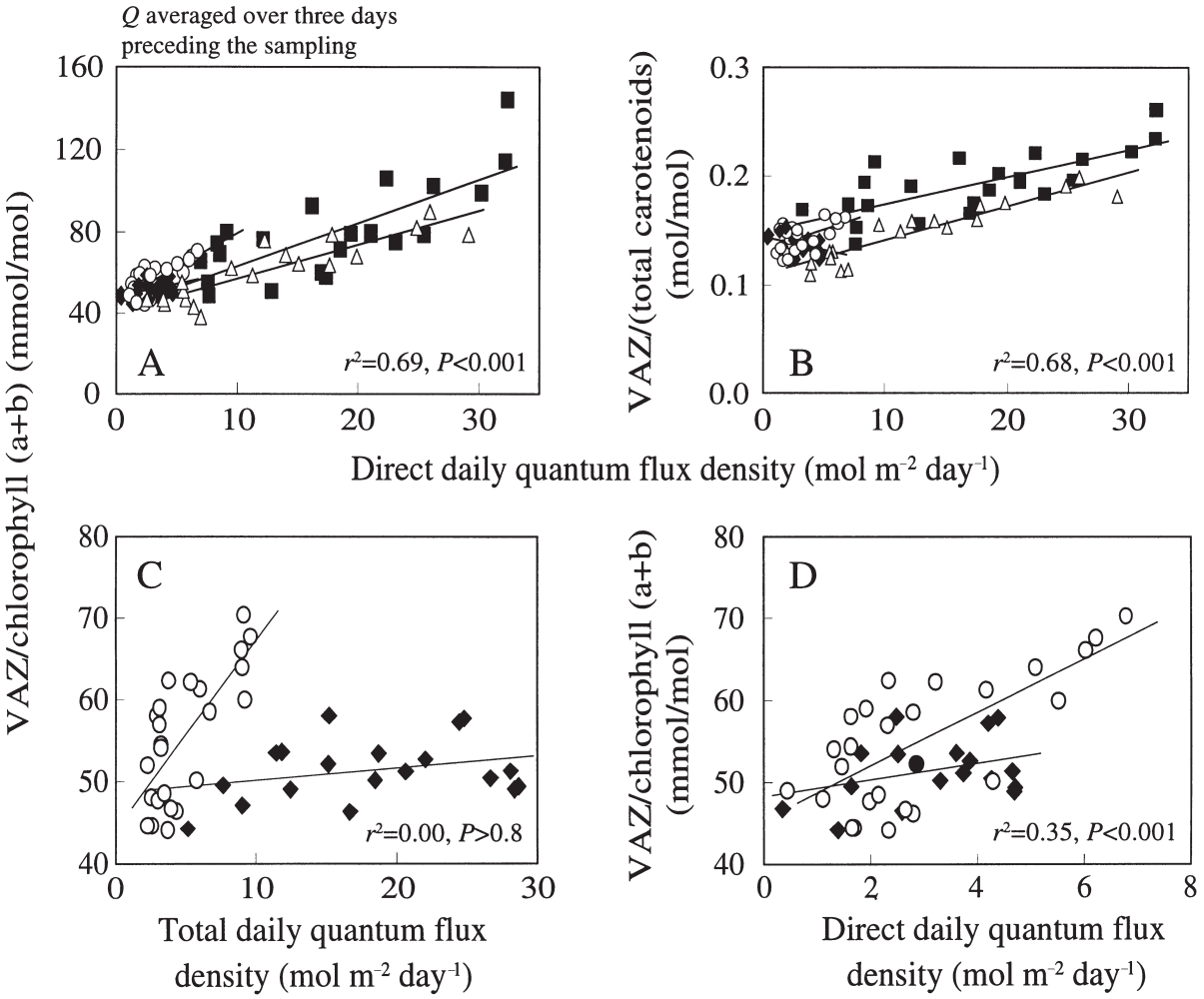

Figure 7. Effects of direct daily quantum flux density averaged over the 3 d preceding foliar sampling (D_{3d}) on VAZ/Chl (a) and VAZ/Car (b) for all samples, and a comparison of the relationships of VAZ/Chl with T_{3d} (c) and D_{3d} (d) in *Tilia cordata*. All r^2s depicted in the figures are for the whole set of values. The coefficients of determination for the regressions within species in (a) and (b) are: *Betula pendula* (△), $r^2 = 0{\cdot}79$, $P < 0{\cdot}001$ for VAZ/Chl and $r^2 = 0{\cdot}86$, $P < 0{\cdot}001$ for VAZ/Car; *Populus tremula* (■), $r^2 = 0{\cdot}61$, $P < 0{\cdot}001$ for VAZ/Chl and $r^2 = 0{\cdot}54$, $P < 0{\cdot}001$ for VAZ/Car; *Tilia cordata* (Järvselja, ○), $r^2 = 0{\cdot}50$, $P < 0{\cdot}001$ for VAZ/Chl, $r^2 = 0{\cdot}30$, $P < 0{\cdot}01$ for VAZ/Car; *T. cordata* (Tartu, ◆), $r^2 = 0{\cdot}14$, $P > 0{\cdot}1$ for VAZ/Chl, $r^2 = 0{\cdot}20$, $P > 0{\cdot}05$ for VAZ/Car.

light (Niinemets & Tenhunen 1997). A similar conclusion may also be derived from our data when both species are pooled (Fig. 3c).

Foliage potential for light harvesting in relation to canopy light gradients

In general, Chl/area is relatively insensitive to canopy light environment [cf. Niinemets (1997b) for a literature review] as was also found in the current study. This constancy of Chl/area results from large changes in Chl/mass with irradiance (Fig. 4b) compensating for the strong positive effects of light on M_A (Fig. 4a). Tight negative hyperbolic dependencies of leaf Chl/mass and Chl/N (e.g. Niinemets 1997b; Niinemets & Tenhunen 1997; Niinemets *et al.* 1998) on growth irradiance have regularly been observed, and improving leaf absorptance per mass, they result in an improvement of light supply to leaf cells in low light (cf. Niinemets 1997b). Because there exists a fairly fixed stoichiometry of chlorophylls and carotenoids in thylakoids, the total leaf carotenoid pool was also strongly correlated with leaf chlorophyll. In earlier studies, the total carotenoid content per dry mass was higher in low than in high light (Lichtenthaler 1971; Czeczuga 1987), and the content of different carotenoids, except for VAZ, was relatively constant when expressed on a surface area basis (Adams *et al.* 1992).

The changes in the foliar chlorophyll *a*/*b* ratio in response to a transfer to different light environments exhibit contradictory trends, which are evidently species dependent (Demmig-Adams *et al.* 1989; Bilger *et al.* 1995a). The fact that chlorophyll content per dry mass as well as the chlorophyll *a*/*b* ratio were better correlated with short-term than with long-term average Q_i (Table 1)

provides indirect evidence that beyond the rapid alterations in the VAZ pool size (cf. below), the stoichiometry and content of other leaf pigments may be adjusted fairly quickly to prevailing light conditions. On the other hand, declining chlorophyll concentrations with increasing light inputs over the short term may also be attributable to chlorophyll photodestruction indicative of inadequate changes in the VAZ pool size or of changes in VAZ that were not rapid enough to avoid photodamage.

Adjustment of the xanthophyll cycle pool size to canopy light gradients

The basic positive relationship between VAZ pool size and integrated Q is independent of whether VAZ is expressed on the basis of leaf area, mass, chlorophyll or total carotenoids (Czeczuga 1987; Thayer & Björkman 1990; Adams *et al*. 1992; Demmig-Adams & Adams 1992a; Brugnoli, Cona & Lauteri 1994; Demmig-Adams & Adams 1994; Königer *et al*. 1995; Logan *et al*. 1996), and reflects increased capacities for harmful dissipation of excess energy at higher light (Bilger *et al*. 1995a). We found correlative evidence that the VAZ pool size acclimated to short-term integrated light (Fig. 5), and in particular, to its direct component (Fig. 7). Given that direct light gives a better estimate of the quantum flux densities which exceed the foliage capacity for photochemical quenching of excitation energy, this difference between total and direct integrated light agrees with the view that the xanthophyll cycle capacity is controlled by the level of excess energy (Bilger *et al*. 1995a; Demmig-Adams *et al*. 1995), and also fits a previous observation that at a common total integrated photon flux, the VAZ pool size is considerably less with conditions of uniform artificial lighting than in natural environments with a strongly fluctuating light climate with high peak intensities (Thayer & Björkman 1990). Due to the higher fraction of excess light, leaves low in photosynthetic capacity generally possess larger VAZ pools (Khamis, Lamaze & Foyer 1990; Bilger *et al*. 1995a; Demmig-Adams *et al*. 1995). However, in the current study, there was a positive relationship between foliar photosynthetic capacity and VAZ pool size (Fig. 6). A similar correlation was found among a number of tropical tree species when changes in photosynthesis occurred in response to a canopy light gradient (Königer *et al*. 1995). In this as well as in our study, the leaves with the highest photosynthetic capacities were also exposed to the highest excess irradiances in the canopy, and this is likely to provide the explanation for this inconsistency with the laboratory studies. Of course, other environmental factors also vary along canopy light gradients. Air temperature increases and humidity decreases, resulting in a greater water vapour pressure deficit with increasing irradiance in the canopy (e.g. Eliáš 1979; Chiariello 1984; Shuttleworth *et al*. 1985), and consequently, in a greater evaporative demand and potential water stress at higher Q_i. Interaction of water stress with light level leads to a greater fraction of potentially damaging excess light (Björkman & Powles 1984; Valladares & Pearcy 1997). Thus, the form of the relationships depicted in Fig. 7 will be the result of a combination of multiple stress factors.

VAZ pool size was negatively related to shade tolerance in 20 temperate herbaceous species (Johnson *et al*. 1993). Unfortunately, in that as well as in several other studies focusing on interspecific differences in VAZ (e.g. Thayer & Björkman 1990; Demmig-Adams & Adams 1992a; Logan *et al*. 1996), the species with higher VAZ were exposed to higher Q_i. Because light environment is a strong determinant of VAZ pool size, the observed interspecific variability may simply have resulted from differences in growth light environment rather than from species-specific potentials to change leaf pigment stoichiometry. In the current study, more shade-tolerant species tended to possess greater VAZ pool sizes (Fig. 7a,b). However, differences in species morphology may also be the cause for this variability. In the canopy studied, leaf blades of *T. cordata* were on average more horizontal (17·7 ± 2·1° with respect to horizontal) than those of *P. tremula* [48·2 ± 3·1°; Niinemets (1998)]. Using these leaf angles in calculations of photon receipt, the leaves of *T. cordata* were exposed on average to 1·4 times greater quantum flux at mid-day than those of *P. tremula* at a similar incident quantum flux on a horizontal surface. As previous studies demonstrate, the VAZ pool size is greater in more horizontal leaves (Adams *et al*. 1992; Lovelock & Clough 1992). Nevertheless, these calculations should be interpreted with caution, because the species studied shared a limited common light range, and within a species leaf angles tend to be more vertical in higher light (McMillen & McClendon 1979).

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