Oecologia (1998) 116:26–37

Hendrik Poorter · John R. Evans

Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area

Received: 11 October 1997 / Accepted: 9 April 1998

Abstract Factors that contribute to interspecific variation in photosynthetic nitrogen-use efficiency (PNUE, the ratio of CO_2 assimilation rate to leaf organic nitrogen content) were investigated, comparing ten dicotyledonous species that differ inherently in specific leaf area (SLA, leaf area:leaf dry mass). Plants were grown hydroponically in controlled environment cabinets at two irradiances (200 and 1000 μmol m^{-2} s^{-1}). CO_2 and irradiance response curves of photosynthesis were measured followed by analysis of the chlorophyll, Rubisco, nitrate and total nitrogen contents of the leaves. At both irradiances, SLA ranged more than twofold across species. High-SLA species had higher in situ rates of photosynthesis per unit leaf mass, but similar rates on an area basis. The organic N content per unit leaf area was lower for the high-SLA species and consequently PNUE at ambient light conditions ($PNUE_{amb}$) was higher in those plants. Differences were somewhat smaller, but still present, when PNUE was determined at saturating irradiances ($PNUE_{max}$). An assessment was made of the relative importance of the various factors that underlay interspecific variation in PNUE. For plants grown under low irradiance, $PNUE_{amb}$ of high-SLA species was higher primarily due to their lower N content per unit leaf area. Low-SLA species clearly had an overinvestment in photosynthetic N under these conditions. In addition, high SLA-species allocated a larger fraction of organic nitrogen to thylakoids and Rubisco, which further increased $PNUE_{amb}$. High-SLA species grown under high irradiance showed higher $PNUE_{amb}$ mainly due to a higher Rubisco specific activity. Other factors that contributed were again their lower contents of N_{org} per unit leaf area and a higher fraction of photosynthetic N in electron transport and Rubisco. For $PNUE_{max}$, differences between species in organic leaf nitrogen content per se were no longer important and higher $PNUE_{max}$ of the high SLA species was due to a higher fraction of N in photosynthetic compounds (for low-light plants) and a higher Rubisco specific activity (for high-light grown plants).

Key words Interspecific variation · Nitrogen · Photosynthesis · Photosynthetic nitrogen use efficiency · Specific leaf area

Introduction

A strong positive correlation has been observed between the light saturated rate of photosynthesis of a leaf and its nitrogen content (Field and Mooney 1986; Evans 1989; Reich et al. 1994, 1995a,b). That is, generally, higher nitrogen contents are associated with higher rates of maximum photosynthesis. The reason for this strong relationship is the large amount of leaf organic nitrogen (up to 75%) present in the chloroplasts, most of it in the photosynthetic machinery (Evans & Seemann 1989). However, despite this strong connection between photosynthesis and nitrogen, the ratio between the rate of photosynthesis and the amount of (organic) nitrogen in the leaf, the photosynthetic nitrogen-use efficiency (PNUE), is not constant. Field & Mooney (1986) reported a three-fold difference in PNUE between annual herbs and evergreen woody species, grown in the field. Subsequently, interspecific variation in PNUE has been described for a wide range of herbaceous species (growth chamber: Poorter et al. 1990; Pons et al. 1994; glasshouse: Boot & Den Dubbelden 1990; field: Mulkey et al. 1991) and trees (glasshouse: Lloyd et al. 1992; field: Reich et al. 1991, 1994, 1995a; Gower et al. 1993), determined either at growth irradiance ($PNUE_{amb}$) or under saturating light conditions ($PNUE_{max}$).

H. Poorter (✉)[1] · J. R. Evans
Environmental Biology, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia

Present address:
[1]Department of Plant Ecology and Evolutionary Biology, University of Utrecht, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands
e-mail: H.Poorter@Bio.uu.nl; Fax: + 31-30-2518366

There is an intriguing correlation between the observed PNUE of a species on the one hand, and its specific leaf area (SLA, leaf area:leaf dry mass) on the other. That is, species with a high SLA almost invariably have a high PNUE (Fig. 1A). SLA is an important determinant of interspecific variation in relative growth rate (Lambers & Poorter 1992; Garnier 1992) and interconnects with a suite of other traits: high-SLA species generally have higher water contents per unit dry mass, lower concentrations of cell wall compounds and C per unit leaf mass, and higher mass based concentrations of N (Poorter & Bergkotte 1992). Thus, the variation in PNUE is linked with a suite of traits that determine the growth potential of a species.

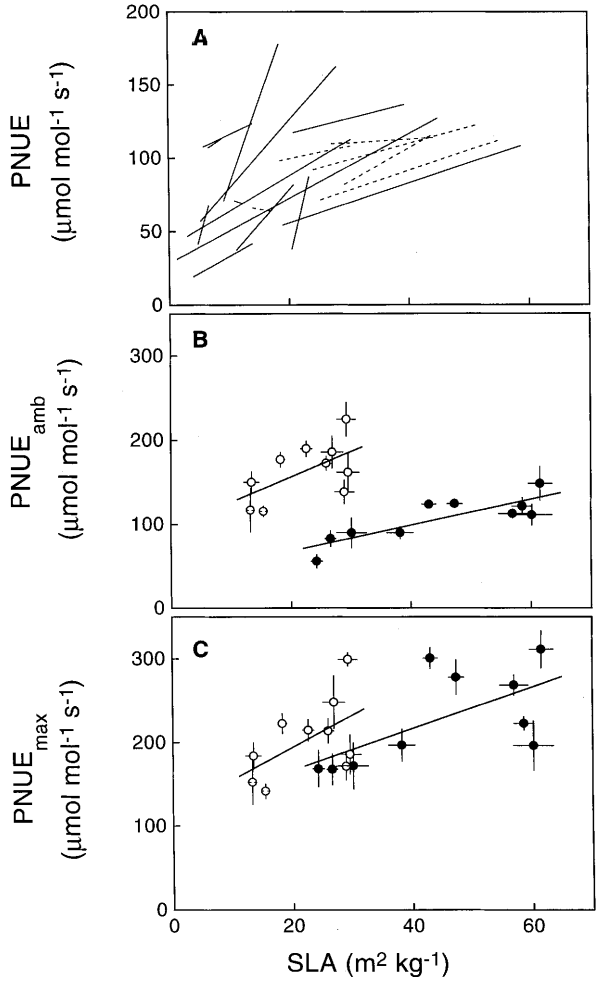

Fig. 1 A Photosynthetic nitrogen use efficiency (PNUE) in relation to inherent variation in specific leaf area (SLA) for a range of experiments with interspecific comparisons. Regression lines were calculated for each data set. *Continuous lines*: $PNUE_{max}$ estimates from Walters & Field 1987; Harrington et al. 1989, Mulkey et al. 1991; Reich et al. 1991; Hollinger 1992, Lloyd et al. 1992; Sheriff 1992; Thompson et al. 1992; Gower et al. 1993; DeLucia & Schlesinger 1995; Reich et al. 1995a, 1997; *broken lines*: $PNUE_{amb}$ estimates from Konings et al. 1989; Boot & Den Dubbelden 1990; Poorter et al. 1990; Pons et al. 1994; Atkin et al. 1996; E. Garnier, O. Gobin, G. Laurent & J. Roy, unpublished work) **B** $PNUE_{amb}$ for species in the present experiment, **C** $PNUE_{max}$ for species of the present experiment, plotted against SLA. In **B** and **C** *open circles* indicate the high-light grown plants (1000 μmol quanta m^{-2} s^{-1}), *closed symbols* the low-light grown plants (200 μmol quanta m^{-2} s^{-1}). Mean values ± SE are given, $n = 8$ for SLA and $n = 4$ for PNUE. Species order in SLA is given in Table 1. Regression lines are calculated for low- and high-light grown plants separately and are drawn *continuous* if significant, *broken* if not significant

Given the strong correlation between PNUE and SLA, and the importance of PNUE in determining the efficiency with which species utilise N to achieve growth (Garnier et al. 1995) the question arises what causes high-SLA species to have higher PNUEs? Several explanations for variation in PNUE have been suggested (Field & Mooney 1986; Evans 1989; Lambers & Poorter 1992; Pons et al. 1994). First, at a given irradiance, there may be a difference between species in the fraction of light absorbed by the leaf. Second, plants may have similar CO_2 response curves of photosynthesis, but operate at a different intercellular CO_2 partial pressure (p_i), or CO_2 partial pressure at the sites of carboxylation within the chloroplasts. Third, there could be variation in the proportion of organic N compounds allocated to photosynthetic versus non-photosynthetic functions. Fourth, CO_2 assimilation rate per unit photosynthetic N may be different, because the plants have partitioned photosynthetic N differently between light harvesting complexes, electron transport and CO_2 fixation. Fifth, there may be variation in the activation state or specific activity of Rubisco. Sixth, there could be a different amount of respiration in the light. Seventh, it may be that all of the above factors are similar, but that due to a different content of N per unit leaf area, there is species-specific variation in the amount of light required to saturate photosynthesis.

In this paper, we analyse which of these suggestions can explain interspecific variation in PNUE in a quantitative way. To this end, ten dicotyledonous plant species which we expected to differ widely in SLA were grown at a low (200 μmol m^{-2} s^{-1}) and a high (1000 μmol m^{-2} s^{-1}) irradiance and the photosynthesis-nitrogen relationships determined. Here we will focus on the interspecific variation in PNUE, separating the 10 species on the basis of their SLA. Data from the two irradiance treatments will be basically treated as a kind of replication. In a separate paper we will analyse the effects of growth irradiance on nitrogen partitioning.

Materials and methods

Growth of the plants

Ten plant species were grown from seeds. These species, all dicotyledons, are listed in Table 1. The seedlings were placed in a growth cabinet with the following conditions: day: 11 h, irradiance either 200 or 1000 ± 30 μmol quanta m^{-2} s^{-1}, temperature 25 ± 0.5°C, relative humidity circa 70%; night: 13 h, temperature 20 ± 0.5°C. The CO_2 partial pressure in the cabinets was maintained at 350 ± 25 μbar. Light was provided by 1000-W Universal Metal Halide lamps (Sylvania, USA). Plants were grown in a frequently replenished modified Hoagland solution with a nitrate concentration of 2 mmol l^{-1} (Poorter & Remkes 1990). The pH was set to 5.8 and regularly maintained.

Experimental design

Two growth cabinets were used for the experiment, each cabinet divided by black shadecloth into a high light and a low light compartment. All plant species were grown once in each cabinet. After plants had developed leaves with a size large enough to be adequately measured (3–12 weeks after germination), four plants were selected per species, treatment and cabinet and measured in one day within the normal light period. CO_2-response curves were obtained on two plants in the gas exchange system, light-response curves on the other two plants with a fluorometer. After the determinations, transmittance and reflectance were measured with a Taylor integrating sphere (Taylor 1935). Subsequently, a number of leaf samples were punched out of each leaf, five of which were deep-frozen in liquid nitrogen and stored at −80°C for subsequent analysis of chlorophyll and Rubisco. For the other punches, as well as the remainder of the leaf, fresh and dry mass were determined. Leaf area and dry mass of the other leaves, as well as mass of stems and roots were also determined. The procedure was repeated independently with four plants from the other growth cabinet.

Physiological analyses

CO_2 and water exchange were measured in the system described by Brugnoli et al. (1988). The youngest fully expanded leaf was selected and placed into a cuvette. After an acclimation period of 0.5–1 hour, gas exchange was measured at growth conditions. Thereafter irradiance was increased to 1000 μmol m^{-2} s^{-1} in the case of low-light grown plants or 2000 μmol m^{-2} s^{-1} for high-light grown plants, and an A:p_i curve was determined, starting with the lowest CO_2 partial pressure.

Steady state fluorescence (Φ_{PSII}, Genty et al. 1989) was first determined under growth conditions with a PAM 101 fluorometer (Walz, Germany). Thereafter, CO_2 partial pressure in the air was raised to 1500 μbar and Φ_{PSII} determined as a function of irradiance from 50 to 2700 μmol quanta m^{-2} s^{-1}. After measuring absorptance, harvesting of the investigated leaf as well as the remainder of the plant was carried out as described above.

Chemical analyses

Chlorophyll was determined according to Porra et al. (1989), on two leaf discs per plant. Rubisco content was assessed with a ^{14}CABP binding method described in Mate et al. (1993) assuming a molecular weight of 550 kd. Total C- and N-content of the samples was determined with a C-H-N analyser (Carlo Erba, model 1106, Milano) using combustion gas chromatography (Pella and Colombo 1973). Nitrate was quantified following the Cataldo (1975) procedure.

Calculations and statistical analysis

Data were analysed with the SPSS statistical package. Light response curves were fitted with a non-rectangular hyperbola (cf. Evans 1987). The equations of Farquhar & Von Caemmerer (1982) were used to fit the A:p_i curves. Rubisco activity, V_{max}, was calculated ignoring CO_2 diffusion limitations within the leaf (see below) as:

$$V_{max} = (A + R_d)\frac{p_i + K_c(1 + O/K_o)}{p_i - \Gamma_*} \quad (1)$$

for data at low p_i. In this equation A is the rate of CO_2 assimilation, K_c and K_o are the Michaelis-Menten constants for Rubisco carboxylase and oxygenase, respectively, Γ_* is the CO_2 compensation point in the absence of non-photorespiratory mitochondrial CO_2 release (R_d) and O is the oxygen partial pressure. R_d was determined from the A:p_i curve near the CO_2 compensation point as the rate at $C_i = \Gamma_*$. Electron transport rate, J, was calculated from data at higher p_i as:

$$J = (A + R_d)\frac{4p_i + 8\Gamma_*}{p_i - \Gamma_*} \quad (2)$$

The values derived for *Nicotiana tabacum* by Von Caemmerer et al. (1994), K_c = 404 μbar, K_o = 248 mbar and Γ_* = 36.9 μbar, were assumed for all species.

To explore the relation between CO_2 transfer conductance from the intercellular spaces to the site of carboxylation (g_w) on calculated Rubisco activity, additional equations are needed (Farquhar & Von Caemmerer 1982):

$$\frac{\delta A}{\delta p_c} = k = E \cdot k_{cat}\frac{\Gamma_* + K_c(1 + O/K_o)}{(p_c + K_c(1 + O/K_o))^2} \quad (3)$$

where the slope of the response of CO_2 assimilation rate to CO_2 partial pressure at the sites of carboxylation, p_c, can be calculated from the Rubisco activity (the product of Rubisco content, E, and Rubisco specific activity, k_{cat}). The parameter k can be converted to the slope of the A:p_i curve near the CO_2 compensation point if one has a value for g_w (Evans 1986):

$$\frac{\delta A}{\delta p_i} = \frac{k}{1 + k/g_w} \quad (4)$$

In this way, V_{max} can be recalculated ignoring CO_2 diffusion limitations within the leaf:

$$V_{max} = \frac{\delta A}{\delta p_i} \cdot \frac{(p_i + K_c(1 + O/K_o))^2}{\Gamma_* + K_c(1 + O/K_o)} \quad (5)$$

Organic N was calculated as total N minus nitrate-N. A common relationship has been observed in *Pisum*, *Spinacea*, *Triticum* and *Alocasia* that links thylakoid nitrogen (N_{P+E}) to the electron transport capacity (H, mmol O_2 (mol Chl)$^{-1}$ S^{-1}) (Evans 1989): $N_{P+E} = (H \times 0.316 + 33.1) \times$ Chl, where N_{P+E} is in moles, Chl is chlorophyll (moles) and $H = J/4/$Chl, the electron transport rate converted to oxygen evolution rate per unit chlorophyll. We further divide thylakoid nitrogen into two fractions, pigment-protein nitrogen (N_P) and that associated with the electron transport chain and photophosphorylation (N_E). Growth irradiance alters the relative abundance of the pigment-protein complexes, as evidenced by a change in Chl a/b ratio. While each species varied slightly in their Chl a/b ratio, it suffices in this study to simply assume a nitrogen cost of 41 or 38.5 mol N (mol Chl)$^{-1}$ for high- and low-light grown plants, respectively (Evans & Seemann 1989). N_E was calculated as the difference between thylakoid N and pigment-protein N.

Data were tested for significant differences ($P < 0.05$) for plants grown at the two irradiances separately in a one-way ANOVA with species as the independent variable. Total sum of squares due to Species was subsequently broken down with orthogonal polynomials, using SLA to characterise the distance between species. In this paper we consider the linear trends only.

Results

The species under investigation were selected under the expectation that they would differ in SLA, regardless of the irradiance in which the plants were grown. Indeed, there was a more than twofold variation between species in SLA, both at low and high light (Table 1, $P < 0.001$ in a one-way ANOVA for both irradiances). Species differences followed differences in life-form, with trees and shrubs having low values and herbaceous species having high values. Concomitantly, the four woody species showed significantly higher C concentrations (36.2 vs.

Table 1 Species used in the experiment, life form, and the specific leaf area (SLA) for leaves used in the photosynthesis measurements of plants grown at low (200 μmol quanta m^{-2} s^{-1}) and high irradiance (1000 μmol quanta m^{-2} s^{-1}). Mean values ± SE (n = 8)

Species	Life form	SLA (m^2 kg^{-1})	
		Low irradiance	High irradiance
Radyera farragei (F. Muell.) Fryxell & Hashmi	Shrub	24.3 ± 0.9	13.2 ± 0.3
Nerium oleander W.	Shrub	26.6 ± 0.9	15.4 ± 0.4
Eucalyptus macrorhyncha F. Muell.	Tree	30.1 ± 2.5	13.4 ± 1.3
Eucalyptus goniocalyx F. Muell. ex Miq.	Tree	38.3 ± 2.2	18.3 ± 0.7
Echium plantagineum L.	Herb	43.1 ± 1.2	22.6 ± 0.9
Plantago major L. ssp. *pleiosperma*	Herb	47.4 ± 1.2	25.9 ± 0.9
Datura stramonium L.	Herb	57.0 ± 2.3	26.9 ± 1.8
Nicotiana tabacum L.	Herb	58.6 ± 1.7	29.6 ± 1.9
Physalis peruvianum L.	Herb	60.2 ± 3.3	28.9 ± 1.6
Raphanus sativus L.	Herb	61.6 ± 2.0	29.3 ± 1.6

30.9 mmol C g^{-1} at high light) as well as lower water contents (4.8 vs. 13.0 g H_20 g^{-1} at high light) per unit leaf mass.

The rates of photosynthesis, measured under growth conditions, varied little between species at low light (Fig. 2A). Interspecific differences became more prominent at high light ($P < 0.05$), but no significant relation with SLA was found. Thus, under both light conditions, high SLA species achieved similar rates of photosynthesis as low-SLA species with a smaller biomass invested per unit area. Consequently, photosynthesis on a mass basis is considerably higher for the high-SLA species (Fig. 2B, $P < 0.001$). Traditionally, rates of photosynthesis are expressed on an area basis. However, interspecific variation in relative growth rate has been shown to be more strongly related to photosynthesis per unit leaf mass, in this way taking into account the variation in SLA (Poorter et al. 1990; Walters et al. 1993). In the graphs to follow we will express variables on a mass basis. However, the plotted trends can easily be evaluated on an area basis by drawing a straight line from the origin. If this line fits the mass-based data well (as for the low-light plants in Fig. 2B) the implication is that there are no differences on an area basis (Fig. 2A). A line through the origin intersecting the low-SLA data at a steeper slope than the high-SLA data implies higher values on an area basis for the low-SLA species (low-light plants in Fig. 2C).

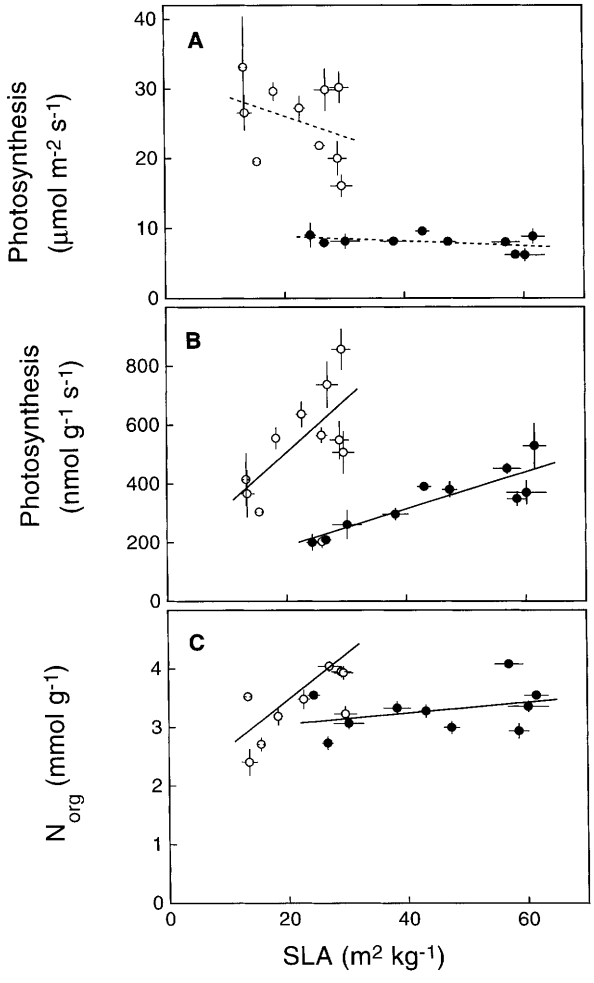

Fig. 2 **A** Rate of photosynthesis measured at ambient irradiance, expressed on an area basis and **B** on a mass basis. **C** Concentration of organic leaf nitrogen on a mass basis. Mean values ± SE (n = 4 for **A** and **B**, n = 8 for **C**). Further information is in the legend of Fig. 1

High-SLA species had significantly higher concentrations of total N and NO_3^- (data not shown). The organic N-concentration is also higher, especially at high irradiance (Fig. 2C). Increases in N_{org} were not as strong as those in photosynthesis. Consequently, $PNUE_{amb}$, determined under growth conditions, was on average 44% higher for the high-SLA species (Fig. 1B, $P < 0.001$). The PNUE at high irradiance, calculated from CO_2 response curves of photosynthesis at 287 μbar (equivalent to the mean p_i for all species) and 1000 or 2000 μmol quanta m^{-2} s^{-1} for the low- and high-light grown plants, respectively, is shown in Fig. 1C. Differences between the low- and high-SLA species were still present, 38% on average ($P < 0.001$).

What physiological or chemical differences cause this systematic variation in PNUE under growth conditions? There were differences between plants grown at high and at low light in leaf reflectance and transmittance (data not shown), but absorptance was rather similar at both irradiances (Fig. 3A, $P > 0.05$). There was a slight but significantly ($P < 0.001$) higher leaf absorptance by the low-SLA species, which was due to significantly higher chlorophyll contents per unit leaf area (data not shown). On a mass basis, chlorophyll was higher for the high-

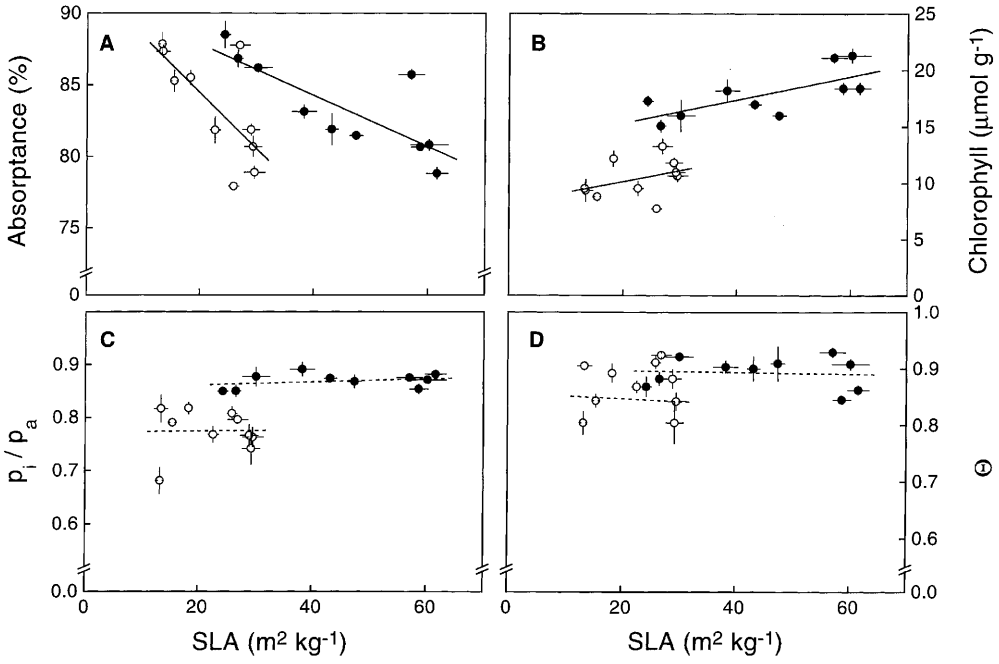

Fig. 3 **A** Leaf absorptance, **B** chlorophyll content per unit leaf mass, **C** the ratio of intercellular to ambient CO_2 partial pressure, and **D** the curvature of the light response curve (Θ) for leaves of 10 different species grown at low (200 μmol quanta m^{-2} s^{-1}, *solid circles*) and high (1000 μmol quanta m^{-2} s^{-1}, *open circles*) irradiance. Mean values ± SE ($n = 8$ for **A** and **B**, $n = 4$ for **C** and **D**). Further information is in the legend of Fig. 1

SLA species (Fig. 3B, $P < 0.001$). There was a difference in p_i/p_a under ambient growth conditions due to irradiance, but no systematic variation with SLA was detected (Fig. 3C, $P > 0.05$).

We did not determine directly how much N was invested in photosynthetic compounds. The amount of N invested in the thylakoids (N_{P+E}) was calculated using the electron transport capacity derived from A:p_i curves and chlorophyll content, assuming all species share the unique relationship established for *Pisum*, *Spinacea*, *Triticum* and *Alocasia* (Evans 1989). Low-SLA species had considerably less N_{P+E} than high-SLA species under both irradiance environments (Fig. 4A). We measured Rubisco content and hence how much N was present in Rubisco (N_R). There was a difficulty determining Rubisco content for *Eucalyptus macrorhyncha* in either irradiance and *E. goniocalyx* at high irradiance, despite taking precautions to avoid protein degradation during extraction. Therefore, for these three cases, Rubisco content was calculated from V_{max} obtained from each A:p_i curve and the average specific activity of Rubisco for all other species in a given growth irradiance (see Fig. 6B). Rubisco content on a mass basis was higher for the high-SLA species (Fig. 4B, $P < 0.001$), with the opposite being true for Rubisco per unit leaf area. The fraction of organic N found in thylakoids plus Rubisco, which is a conservative estimate of photosynthetic nitrogen that does not include N invested in other Calvin and photorespiratory cycle enzymes, is given in Fig. 4C. The proportion was greater in high- than in low-SLA species, especially at low irradiance (51 vs. 41%, $P < 0.001$).

Partitioning of photosynthetic N between light capture, electron transport/photophosphorylation and Rubisco is shown in Fig. 5. High-SLA species invested proportionally less N in pigment-protein complexes than low-SLA species (Fig. 5A, $P < 0.001$). Conversely, high-SLA plants invested relatively more in Rubisco than did low-SLA species, at low but especially at high light (Fig. 5C, $P < 0.05$ and $P < 0.001$, respectively). At low light, high-SLA species had modestly higher proportions of N invested in electron transport capacity and Rubisco. The balance between N investment in electron transport and Rubisco capacities can also be derived from the ratio J/V_{max}. The ratio J/V_{max} declined slightly but significantly with increasing SLA, with the ratio being consistently greater for high-light than low-light grown plants (Fig. 6A). The very high J/V_{max} ratio for high-light grown *Radyera* was due to peculiar A:p_i curves that could not be fitted well by the electron transport limited equation.

We did not measure Rubisco activation state. The main source of variation appears to simply be that the greater the Rubisco content per unit area, the lower the in vivo specific activity, hence the high growth irradiance treatment had lower in vivo specific activity (Fig. 6B). Because the low SLA group had greater Rubisco contents per area, this also lead to a lower Rubisco specific activity than in the high SLA group. The respiration rate in the light per unit leaf area was significantly greater in the low-SLA species (Table 2). On a mass basis, there was a significant increase in R_d at low light only (Fig. 6C, $P < 0.05$).

Discussion

SLA and CO_2 assimilation

There was a clear difference in leaf traits between the tree seedlings and shrubs on one hand, and the herbaceous species on the other. The former group had lower

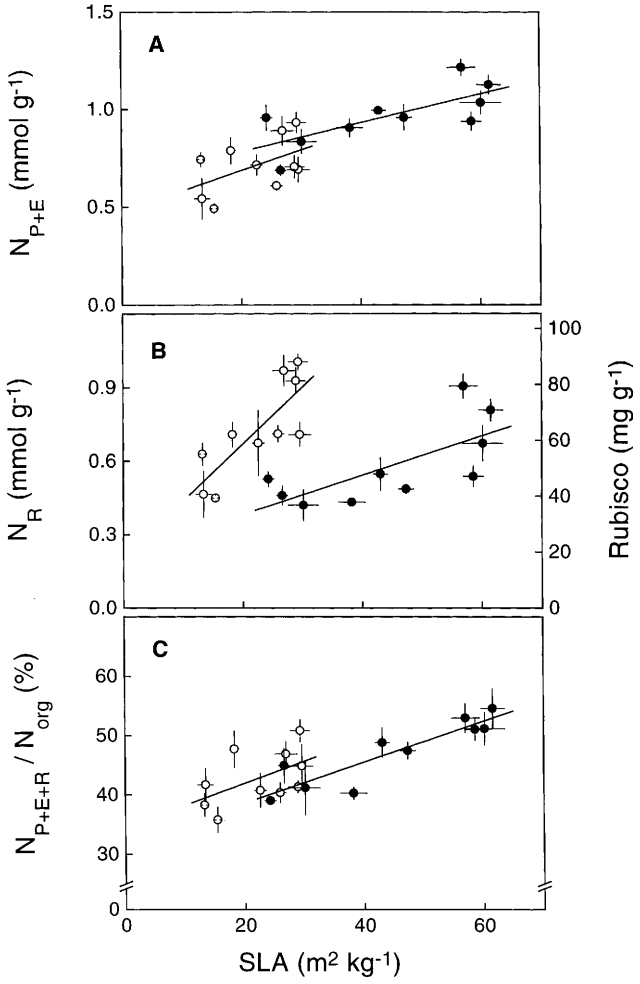

Fig. 4 **A** Thylakoid N content per unit leaf mass, **B** Rubisco content per unit leaf mass, **C** percentage of organic N invested in thylakoids plus Rubisco of 10 different species grown at low (200 μmol quanta m^{-2} s^{-1}, *solid circles*) and high (1000 μmol quanta m^{-2} s^{-1}, *open circles*) irradiance. Mean values ± SE (n = 4). Rubisco contents for *Eucalyptus macrorhyncha* and *E. goniocalyx* (high-light grown) are not based on ^{14}CABP binding but were estimated from V_{max} determined from A:p_i curves and the average Rubisco specific activity for all the species at that growth irradiance. Further information is in the legend of Fig. 1

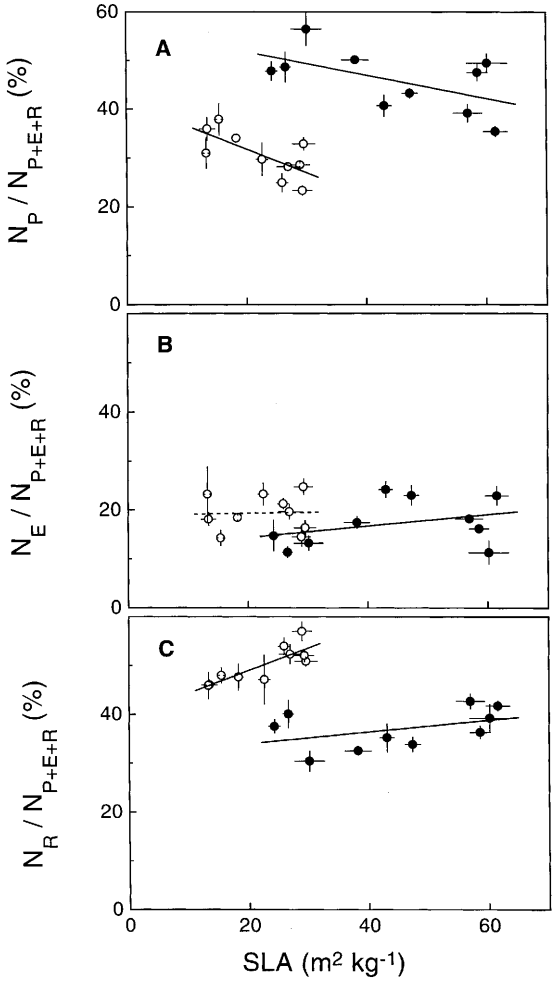

Fig. 5 Percentage of the N in thylakoids plus Rubisco invested in **A** pigment-protein complexes, N_P **B** electron transport/ photophosphorylation complexes, N_E, and **C** Rubisco, N_R, for 10 different species grown at low (200 μmol quanta m^{-2} s^{-1}, *solid circles*) and a high (1000 μmol quanta m^{-2} s^{-1}, *open circles*) irradiance. Mean values ± SE (n = 4). Further information is in the legend of Fig. 1

specific leaf areas (Table 1), higher C concentration per unit leaf mass and a lower water content. Such systematic differences have been observed between fast- and slow-growing herbs (Poorter & Remkes 1990; Van der Werf et al. 1993) and between fast- and slow-growing tree species (Reich et al. 1991, 1995b; Walters et al. 1993). No systematic differences in the rate of photosynthesis per unit leaf area were observed, but as the leaf area:total plant mass ratio was much higher for the herbaceous species, total estimated carbon gain per day was on average 60 and 80% higher for these species, grown at low and high light respectively (data not shown). As the low-SLA species have higher C concentrations (see Results; cf. Poorter & Bergkotte 1992) and a higher proportion of daily fixed C spent in respiration (Poorter et al. 1990, Walters et al. 1993), we expect the difference in growth rate between the two groups to be even more pronounced than that derived from the carbon gain per total plant alone. The difference in estimated daily carbon gain between the two groups of species coincided with the time required for these plants to gain enough size to be measured in the photosynthesis system. Therefore, although we did not determine the relative growth rate of these species directly, it is clear that the differences in SLA in these plants interconnect with the aforementioned suite of traits that determine a species' potential growth rate.

The high-SLA species showed a higher $PNUE_{amb}$ than the low-SLA ones, when grown at both high and low light. As such, these differences coincide with what seems to be a general trend (Field & Mooney 1986; cf. the literature data compiled in Fig. 1A). What factors cause the interspecific variation in PNUE? First, a higher PNUE may be caused by a relatively high absorption of light. Such a difference will not have any

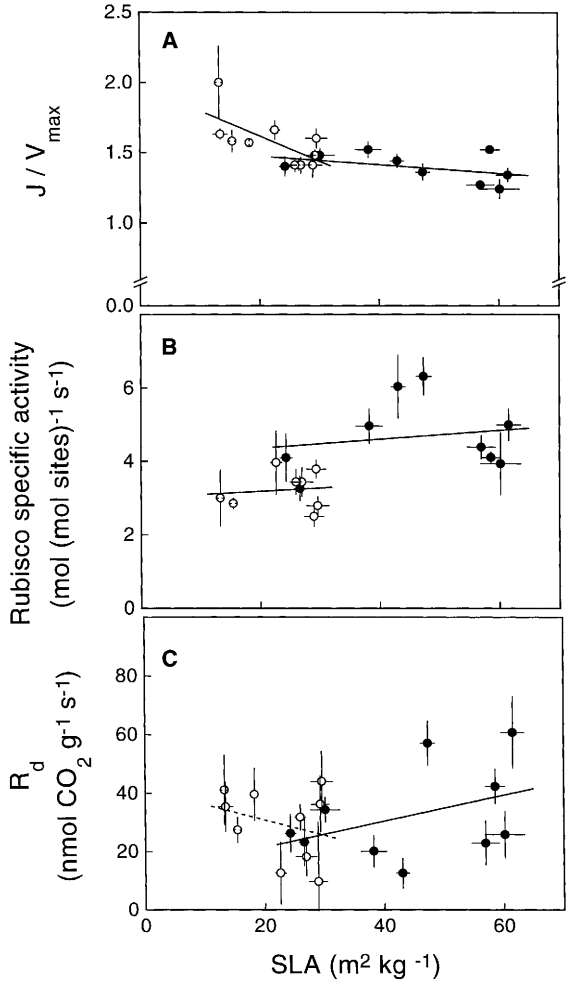

Fig. 6 **A** Ratio of electron transport capacity to Rubisco activity, J/V_{max}, calculated from A:p_i curves, **B** calculated in vivo Rubisco specific activity, **C** respiration during the day expressed on a mass basis, of 10 different species grown at low (200 μmol quanta m^{-2} s^{-1} *solid circles*) and high (1000 μmol quanta m^{-2} s^{-1}, *open circles*) irradiance. Mean values ± SE (n = 4). Further information is in the legend of Fig. 1

effect at saturating light conditions, but in the growth conditions used here may contribute to a higher $PNUE_{amb}$. Leaves of low-SLA species absorbed a small, but significantly higher proportion of light (Fig. 3A). This was due to the higher chlorophyll content per unit area, given the strong relationship between chlorophyll content and absorptance (Evans 1996). However, as the $PNUE_{amb}$ of these species is actually lower, other factors must be involved.

A second reason for a higher PNUE may be that, for a given CO_2 response curve of photosynthesis, high-SLA species operate at a higher CO_2 partial pressure within the leaf. The p_i/p_a showed no systematic variation between high- and low-SLA species (Fig. 3C). Measured at an intermediate light intensity, Poorter & Farquhar (1994) found no systematic difference between fast- and slow-growing herbaceous species either, and this was also found by Pons et al. (1994) in their analysis of four grass species. However, intercellular CO_2 partial pressure does not necessarily indicate the partial pressure of CO_2 at the sites of carboxylation (Von Caemmerer & Evans 1991; Evans & Von Caemmerer 1996). From the literature it seems that the drawdown in CO_2 partial pressure between these two sites is similar in mesophytic and sclerophytic leaves (Von Caemmerer & Evans 1991; Loreto et al. 1992; Evans & Von Caemmerer 1996). This is because in sclerophytic leaves, lower rates of CO_2 assimilation per unit leaf area are associated with low internal CO_2 conductances and the drawdown is the ratio of the two. The significance of the drawdown is clearly evident in the present data from the curvilinear relationship between calculated V_{max} and extracted Rubisco content (Fig. 7). Assuming an equal activity per unit Rubisco for all species, one would expect a direct proportionality between the two, the slope being equal to the specific activity. Curvature is introduced into the relationship when the CO_2 transfer conductance within leaves, g_w, does not change in direct proportion to any change in Rubisco content. We are aware of only two data sets where CO_2 transfer conductance and Rubisco content have been measured over a range of Rubisco contents (*Triticum*, Von Caemmerer & Evans 1991; *Nicotiana*, Evans et al. 1994). For both species, CO_2 transfer conductance increased more slowly than Rubisco content ($g_w = 0.33 + 0.0028\ R$, $r^2 = 0.28$, $n = 15$, see inset Fig. 7). Taking that relationship and assuming a constant in vitro specific activity for Rubisco [6 mol CO_2 (mol sites)$^{-1}$ s^{-1}] across species, one predicts the solid curve in Fig. 7 that describes the present data well, on average. These data suggest that slow-growing species, which have higher Rubisco contents per unit area, should have lower calculated in vivo specific activities, possibly as a result of relatively greater drawdowns from p_i to p_c. As this is at variance with data in the literature (Evans & Von Caemmerer 1996), more work is needed to specifically address this issue. In addition to the uncertainty about diffusion limitations within the leaf, it is also possible that the in vitro specific activity of Rubisco varies between species.

N partitioning

A third reason for a high PNUE can be that some species invested relatively more of their N in photosynthetic machinery. Thylakoid nitrogen was calculated assuming all species share a common relationship with electron transport capacity (Evans 1989). Given the limited number of species (four) that were used in its derivation, it is certainly possible that a given species may not be well described by it. However, this awaits the quantification of thylakoid preparations from other species. Unfortunately, species surveys that have examined variation in electron transport capacity per unit chlorophyll have not measured thylakoid nitrogen content (e.g. Murchie & Horton 1997). Rather than do this, other authors seem to have accepted the general

relationship and instead derive the nitrogen sub-fractions in different ways. Strong relationships have been observed between electron transport rate and both cytochrome *f* and ATPase content (Evans 1987, 1988). Pons et al. (1994) used the nitrogen cost derived by Evans & Seemann (1989) to convert the electron transport rate directly to a nitrogen equivalent to arrive at N_E. Another approach (Hikosaka & Terashima 1995; Niinemets & Tenhunen 1997) has been to also link the photosystem II reaction centre content to electron transport rate, which allows both N_P and N_E to be calculated from the electron transport rate per unit chlorophyll. Since the fundamental data is common to all these cases, the nitrogen costs are necessarily similar.

High-SLA species not only had higher investments in thylakoid N per unit leaf mass, also the amount of Rubisco was higher (Fig. 4A, B). As the increase in total organic N with SLA was less substantial, the fraction of total organic N invested in thylakoids plus Rubisco was higher for the high-SLA species (Fig. 4C). There are not many data to compare these values with. In an analysis of four grass species varying in RGR and SLA, investment in photosynthetic machinery was similar between the two slow-growing and the two fast-growing species (Pons et al. 1994).

Fourth, partitioning of photosynthetic N between light-harvesting, electron transport and Rubisco may differ. Low-SLA species indeed invested more of their photosynthetic N in light-harvesting, both at low as well as high light (Fig. 5A). This was at the cost of the fraction invested in Rubisco at high light, and in both electron transport capacity and Rubisco at low light. Poorter et al. (1990) found a general decline in the ratio of chlorophyll to total leaf N with increasing RGR

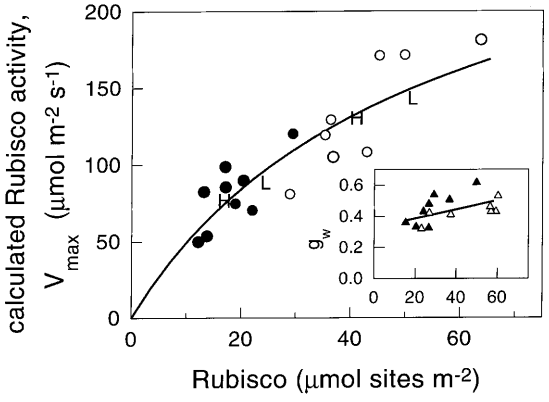

Fig. 7 Calculated Rubisco activity, V_{max}, versus Rubisco content determined by CABP binding to crude leaf extracts. Plants were grown at low (200 μmol quanta m^{-2} s^{-1}, *solid symbols*) and high (1000 μmol quanta m^{-2} s^{-1}, *open symbols*) irradiance, with low- and high-SLA species shown with their mean values (*L* and *H*) for each irradiance treatment. The *solid curve* is calculated assuming the relationship between CO_2 transfer conductance and Rubisco: g_w (mol m^{-2} s^{-1} bar^{-1}) = 0.33 + 0.0028 Rubisco (μmol sites m^{-2}) (*inset*) derived from wheat (*open triangles*, Von Caemmerer and Evans 1991) and tobacco (*solid triangles*, Evans et al. 1994) (see text) and an in vitro Rubisco specific activity of 6 mol (mol sites)$^{-1}$ s^{-1}

across 24 species, that was smaller but still present when one discounted leaf nitrate content. In the present data, chlorophyll per unit organic nitrogen was lower for the high-SLA species, but only in the high growth irradiance treatment. The low-SLA evergreen species, *Flindersia* and *Argyrodendron*, had lower V_{max}/N than the deciduous higher-SLA *Toona* plants (Thompson et al. 1988, 1992). Similarly, V_{max}/N was much lower for low SLA *Citrus* leaves than for deciduous *Prunus* leaves (Lloyd et al. 1992). Therefore it is likely that the proportion of photosynthetic N in light-harvesting is higher and that in Rubisco is lower in the low-SLA species compared to high-SLA species. This generalisation for dicots contrasts with the monocot data of Pons et al. (1994), who found no difference in N invested in photosynthetic functions between the two faster- and two slower-growing grass species.

Partitioning of N between electron transport and Rubisco capacity can also be derived from the ratio of *J* and V_{max}. This ratio was first highlighted by Von Caemmerer and Farquhar (1981). It defines the CO_2 partial pressure where these two processes co-limit CO_2-fixation. The ratio was found to be strongly conserved within a species when photosynthetic capacity differed due to mineral nutrition or age (Von Caemmerer & Farquhar 1981; Evans 1983). We found low-SLA, slow-growing species to have a slightly higher J/V_{max} (Fig. 6A). In a literature survey, Wullschleger (1993) found a strong positive correlation between *J* and V_{max}, with J/V_{max} being slightly greater for perennial versus annual groupings. Given that perennial species have lower SLA and growth rates than annual ones (Garnier 1992), the higher J/V_{max} ratio for the perennial group in Wullschleger's compilation is in line with the present experiment. In addition, J/V_{max} was greater in high-light grown leaves compared to low-light grown leaves as has been found for *Pisum* (Evans 1987) and a few other species (Evans 1988).

Fifth, differences in PNUE could be due to variation in activation state or specific activity of Rubisco. Because of the difficulty of extracting Rubisco from the *Eucalyptus* leaves, we were unable to reach a firm estimate of variation in Rubisco specific activity between high- and low-SLA species. Since photosynthesis in high-light grown plants was Rubisco-limited, a small increase in Rubisco specific activity (Fig. 6B) could partly explain the difference in PNUE at high light.

Although there was species-specific variation in R_d, trends with SLA were absent (high light) or only weak (low light, Fig. 6C). This is in line with data of Villar et al. (1995), who found no significant difference in R_d between an evergreen and a deciduous shrub. However, expressed on an area basis, differences become pronounced, with the low-SLA species having higher values (Table 2), again in line with data on the two shrubs (R. Villar, personal communication). Thus, R_d contributes to some extent to the variation in PNUE.

The final factor that could influence PNUE is a variation in the amount of light needed to saturate

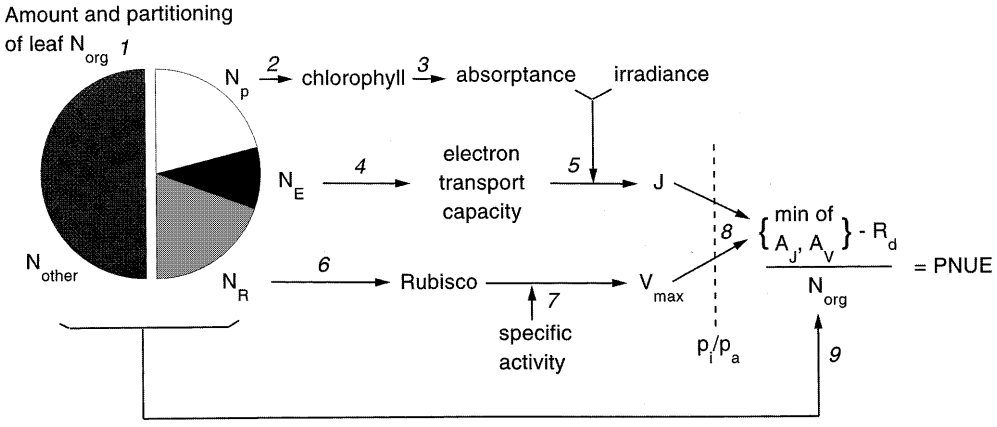

Fig. 8 Scheme for the model to assess the quantitative importance of the various factors influencing PNUE

photosynthesis. To derive Rubisco and electron transport capacities, we measured low- and high-light grown plants at 1000 and 2000 μmol quanta m^{-2} s^{-1}, respectively. It is possible that leaves with greater photosynthetic capacities were underestimated by using a common irradiance. We can assess that from the irradiance response curves that were made by measuring chlorophyll fluorescence. While it has been argued that the fluorescence signal derives from chloroplasts near the surface and thus may be unrepresentative of the leaf as a whole, comparisons between techniques have generally found good agreement between fluorescence and gas exchange techniques (e.g. Genty et al. 1989; Ögren & Evans 1993). The curvature factor Θ describes how abruptly the irradiance response curve saturates. If the irradiance response curve is measured with the leaf oriented incorrectly with respect to the light source, large variations in Θ can be found (Ögren & Evans 1993). There was no dependence of Θ on SLA for either light treatment (Fig. 3D). We estimated the ratio of photosynthetic rate at 1000/2000 and 2000/3000 μmol quanta m^{-2} s^{-1} for the low- and high-irradiance treatments, respectively (fluorescence had been measured up to 2700 μmol quanta m^{-2} s^{-1}). The ratios were always about 0.95 and independent of SLA (data not shown) so we are confident that the estimates of photosynthetic capacity that are presented are a true reflection of the real capacity. If a leaf were to distribute its photosynthetic capacity differently from the way in which light was absorbed, less efficient use would be made of it. There are limited data available on profiles of photosynthetic properties through leaves (e.g. Terashima & Inoue 1985; Nishio et al. 1993) and no data on light absorption profiles, although estimates have been calculated (Evans 1995). We can only compare shade- and sun-grown spinach leaves, where there are differences in SLA, chlorophyll and Rubisco contents. The profiles of relative Rubisco content per layer versus calculated absorbed light are similar in both leaf types and the irradiance response curves generated from these profiles have similar Θ values. Thus, on the basis of this limited evidence and the fact that Θ was independent of SLA, we conclude that inefficient distribution of photosynthetic resources with respect to light absorption through the leaf is not a factor contributing significant variation in PNUE between the species measured here.

Sensitivity analysis

The above analysis shows that several components contribute to variation in PNUE. However, they will not exert the same quantitative effect. To evaluate the relative importance of each of the factors, we constructed a model which incorporated all of the factors listed above (Fig. 8). For a given leaf organic nitrogen content, the amount of nitrogen in Rubisco and thylakoids was first calculated from the observed fraction N_{P+E+R} (indicated as *1* in Fig. 8). This pool was then split into pigment-protein N (N_P), electron transport/photophosphorylation N (N_E) and Rubisco N (N_R). Dividing pigment-protein N by the nitrogen cost per unit chlorophyll (Evans & Seemann 1989) yielded the chlorophyll content of the leaf (*2*) and leaf absorptance (*3*; Evans 1996). Electron transport capacity was given by N_E multiplied by 16.28 mmol e^- (mol $N_E)^{-1}$ s^{-1} (*4*; Evans & Seemann 1989). Subsequently, electron transport rate was then calculated from the irradiance response function (*5*; data not shown). Rubisco activity was given by converting N_R to protein (*6*) and multiplying this by the specific activity (*7*). The actual rate of CO_2 assimilation was calculated at a given p_i/p_a ratio and R_d as the minimum of A_J and A_V , (*8*; cf. Farquhar & Von Caemmerer 1982). PNUE was then the ratio of CO_2 assimilation rate divided by organic leaf nitrogen content (*9*). To test the importance of each character in altering PNUE, the value of a character for the average of the four low-SLA species replaced that of the six high-SLA species with all other characters remaining as the value for high-SLA species. This enabled us to assess the proportion of the total difference in PNUE between the two groups, attributable to each factor (Table 2). This is under the assumption that the differences explained by the several factors are more or less additive. First, the effect of p_i/p_a was analysed. Second, the fraction of organic N invested in thylakoids plus Rubisco

Table 2 The average PNUE and related characteristics for the low- and high-SLA groups grown at low or high irradiance and a sensitivity analysis to assess the relative importance of each of these factors in explaining the difference in PNUE. $PNUE_{amb}$ is defined as the rate of CO_2 assimilation measured under the growth irradiance (200 or 1000 µmol quanta $m^{-2}\,s^{-1}$) per unit leaf organic nitrogen, $PNUE_{max}$ as the rate of CO_2 assimilation measured under 1000 or 2000 µmol quanta $m^{-2}\,s^{-1}$ (low and high-light grown treatments, respectively) per unit leaf organic nitrogen. $PNUE_{amb}$ expl (%) stands for the percentage of the difference in $PNUE_{amb}$ between the low- and the high-SLA species explained by substituting a given character for the high SLA group with the value for the low SLA-group. $PNUE_{max}$ expl (%): idem at saturating irradiance. Values for all parameters are printed in italics, with the most important factor being underlined. A negative value means that changing the character resulted in a PNUE that exceeded that of high SLA group of species

	Growth light (µmol quanta $m^{-2}\,s^{-1}$)	Species group	$PNUE_{amb}$	$PNUE_{max}$	p_i/p_a	N_{P+E+R}/N_{org} (%)	N_P/N_{P+E+R} (%)	J/V_{max}	Rubisco specific activity (mol (mol sites)$^{-1}$ s^{-1})	R_d (µmol $m^{-2}\,s^{-1}$)	N_{org} (mmol m^{-2})
mean	200	low SLA	80	176	0.87	41	49	1.45	4.10	0.92	112
mean	200	high SLA	124	263	0.87	51	42	1.36	4.96	0.69	63
$PNUE_{amb}$ expl (%)					*0*	*24*	*4*	*−3*	*0*	*6*	*85*
$PNUE_{max}$ expl (%)					*0*	*56*	*32*	*−12*	*21*	*4*	*7*
mean	1000	low SLA	130	175	0.78	42	33	1.78	2.93	1.89	200
mean	1000	high SLA	179	222	0.77	43	28	1.48	3.32	0.91	136
$PNUE_{amb}$ expl (%)					*−1*	*16*	*29*	*6*	*39*	*17*	*32*
$PNUE_{max}$ expl (%)					*−1*	*16*	*31*	*23*	*50*	*14*	*−4*

(N_{P+E+R}) was varied. Third, leaf absorptance was altered by varying the fraction of pigment-protein N at the expense of N_E and Rubisco N, while maintaining the ratio of electron transport to Rubisco capacities, J/V_{max}, constant. Fourth, J/V_{max} (and thus N_E relative to N_R) was varied while holding the fraction of pigment-protein N constant. Fifth, Rubisco specific activity was varied, which changes V_{max} and hence J/V_{max}. Sixth and seventh, R_d and total organic leaf N content were altered.

The outcome of the sensitivity analysis depends on the conditions under which the plants are grown and the light intensity at which PNUE is determined (Table 2). The higher $PNUE_{amb}$ of high-SLA species, grown under low-light conditions, is mainly due to their much lower N content per unit leaf area. Given the low-light environment, low-SLA species have a considerable overinvestment in N which cannot be used under those conditions. Under these circumstances, photosynthetic rate is set by the product of quantum yield and absorbed irradiance. Once photosynthetic capacity exceeds this rate, no further gain can be achieved from an extra N investment and $PNUE_{amb}$ necessarily declines with further increases in capacity. For low-light grown plants measured at saturating light, the overinvestment does not play a role anymore. In that case the higher proportion of N invested in thylakoids and Rubisco by the high-SLA species is the most important factor contributing to their higher $PNUE_{max}$. These results are in line with those of Pons et al. (1994) for four grasses. The second most important factor is that high-SLA species allocated less nitrogen to pigment-protein complexes (N_p) and more to electron transport/photophosphorylation complexes (N_E) and Rubisco (N_R).

Low-SLA species grown at high light and measured at ambient conditions show some overinvestment in total N as well, but would also profit from a higher investment in Rubisco and higher activity of that Rubisco. Apparently, these plants are always Rubisco limited. Measured at saturating irradiance, no sign of overinvestment in total leaf nitrogen is seen. The relatively higher $PNUE_{max}$ of the high-SLA species is explained almost completely by their relatively higher Rubisco amount and activity. In this case the higher investment comes at the expense of both the fraction of N invested in light harvesting and in electron transport. High-SLA species also had slightly lower rates of respiration in the light which also increased their PNUE by around 15%. Since p_i/p_a was independent of SLA at either growth irradiance, it did not cause any variation in PNUE.

The model on which the sensitivity analysis is based is a simplified one, with a number of relations and parameters assumed to be in common for both high- and low-SLA species. The most important assumptions are a common relation for all species between chlorophyll content per unit area and absorptance, a fixed relation between J and N_e, and a common Γ^*. There was no indication of SLA influencing the relationship between chlorophyll content and absorptance. Variation in the

biochemical costs and kinetic parameters within the likely biological ranges did not affect the above conclusions either. This is because PNUE is defined as CO_2 assimilation rate divided by total leaf nitrogen. Any change in the biochemical parameters result in changing the fraction of leaf nitrogen in Rubisco and thylakoids at the expense of the other fraction, without altering the assimilation rate per unit total leaf nitrogen.

Conclusions

Species with an inherently high SLA were found to have a higher PNUE, both at low and high growth irradiance and at saturating light. At low irradiance, the low $PNUE_{amb}$ of low-SLA, slow-growing species is caused by their higher organic N contents per unit leaf area, which cannot be fully used in photosynthesis because light is so limiting. For plants grown and measured at a high irradiance, the difference in $PNUE_{amb}$ can be explained mainly by the fact that high-SLA, fast-growing species allocated more N to Rubisco, which tended to show a higher catalytic activity.

Acknowledgements We would like to thank Marilyn Ball for partly providing the necessary equipment, and Habiba Gitay, Jon Lloyd and Jim Virgona for providing part of the seed. Sue Wood, Barbara Setchell and Yvonne van Berkel helped with the chemical analyses. Peter Reich, Riki van den Boogaard and Suzanne von Caemmerer made helpful comments on a previous version of this ms. This investigation was supported by the Netherlands Organization for Scientific Research.

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