Is plasticity in partitioning of photosynthetic resources between and within leaves important for whole-plant carbon gain in canopies?

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Summary

1. The significance for whole-plant carbon gain of plasticity in between-leaf and within-leaf partitioning of photosynthetic resources was investigated using an experimental and modelling approach. *Lysimachia vulgaris* L. was grown at two contrasting stand densities and two levels of nutrient availability in a glasshouse. Whole-plant daily C gain was calculated for the four treatments. The importance of the two forms of plasticity in photosynthetic resource partitioning was investigated by switching distribution patterns of leaf nitrogen and photosynthetic capacity per unit chlorophyll between plants in the two stand densities and recalculating whole-plant C gain, which was used as a measure of fitness.

2. The plants had a high photosynthetic capacity per unit leaf area in top leaves in the dense stands, and a low capacity in bottom leaves. The distribution over plant height was more homogeneous in the open stands. This plasticity in between-leaf resource partitioning was not very important for whole-plant C gain, provided the plants had a dense-canopy type of partitioning. It is argued, however, that this result of the model calculations is valid only for low, but not high, nutrient availability.

3. Photosynthetic capacity per unit chlorophyll, a parameter representative of within-leaf partitioning of photosynthetic resources between capacity and light harvesting, also showed plasticity in response to stand density. High values were found in open stands and in the top leaves in dense stands, whereas a low capacity per unit chlorophyll was found in shaded bottom leaves in dense stands. Plasticity in this trait was also not very important for C gain of plants in stands of contrasting densities. Here the condition was that plants have a distribution pattern found for open-stand plants. However, in the case of subordinate plants that have all their leaves in the shade cast by their taller neighbours, adjustment of photosynthetic capacity per unit chlorophyll to the level of irradiance is much more important for whole-plant C gain.

Key-words: adaptive plasticity, canopy density, light gradient, photosynthetic acclimation, photosynthetic capacity

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Introduction

Phenotypic plasticity in plant traits is considered to be adaptive when a phenotype evoked by a new environment increases fitness compared with that of the original phenotype. However, evidence for the adaptive value of plasticity is often indirect. Few studies have shown experimentally that plasticity does enhance fitness across the range of environmental conditions that a plant may encounter; induction of synthesis of costly defence compounds is one example (Baldwin 1998). A relevant example in this study of canopy density effects is the phytochrome-mediated shade-avoidance response, where plasticity was manipulated genetically and by using experimental lighting systems (Dudley & Schmitt 1996; Ballaré *et al*. 1997; Schmitt 1997; Schmitt, Dudley & Pugliucci 1999). At low density, a plastic response of internode elongation to stand density was superior to a constitutive tall, high-density phenotype, and, at high density to a constitutive short, low-density phenotype. However, it has not been possible so far to produce such phenotypes for the partitioning of leaf N, which precludes an experimental approach for the

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evaluation of adaptive plasticity for this trait. The alternative adopted here is the combination of an experimental and a modelling approach, where the consequences of the phenotype found in one environment for performance in another environment, and *vice versa*, have been evaluated.

A large fraction of leaf nitrogen is involved in the photosynthetic apparatus, and most of that in the part that determines photosynthetic capacity, at least in fast-growing herbaceous plants (Evans 1989a; Evans & Seemann 1989). Leaf N can thus be used as a proxy for photosynthetic capacity (photosynthetic rate at saturating irradiances). Models show that a high leaf N concentration and associated capacity in upper leaves at the expense of lower leaves in dense canopies increases whole-plant carbon gain compared to a uniform distribution in which the leaf N content of all leaves equals the mean (Field 1983; Hirose & Werger 1987b; Pons *et al*. 1989; Schieving *et al*. 1992; Evans 1993b; see reviews by Anten, Hikosaka & Hirose 2000; Kull 2002). Increasing leaf N gradients with increasing canopy density have been found (Hirose *et al*. 1988; Schieving *et al*. 1992; Pons *et al*. 1993; Anten *et al*. 1998). Hence the adaptiveness of plasticity in between-leaf partitioning of photosynthetic resources seems evident. However, apart from the fact that this has not been proven experimentally, two other criticisms can be made. First, enhancement of C gain in canopies is generally concluded by comparing plants with a gradient in photosynthetic capacity with plants with a uniform distribution, whereas plants outside canopies often have a capacity gradient as well, although less steep (Anten *et al*. 1998). Second, arguments are based implicitly on the improvement of C gain when leaf N distribution in crowded conditions is changed from an open-canopy type to a dense-canopy type. However, improvement of photosynthetic performance in non-crowded conditions when changing a dense-canopy to an open-canopy phenotype is questionable, because photosynthesis is not very sensitive to leaf N distribution under these conditions (Field 1983; Schieving *et al*. 1992). The adaptive significance of plasticity in between-leaf photosynthetic resource partitioning is thus not straightforward, because it is not clear to what extent under non-crowded conditions photosynthesis of the open-canopy phenotype exceeds that of the dense-canopy phenotype.

Plasticity also occurs in within-leaf partitioning of resources between the two main functions of the photosynthetic apparatus that are relevant in this context: light harvesting and capacity (Terashima & Evans 1988; Evans 1989b; Evans 1993a; Pons & Pearcy 1994; Anderson, Chow & Park 1995; Hikosaka 1996; Pons & Jordi 1998). More investment in light harvesting improves photosynthetic performance in low-light conditions, and more investment in capacity increases photosynthesis in high-light conditions (Evans & Seemann 1989; Hikosaka & Terashima 1996). Partitioning of photosynthetic resources in leaves is often

close to optimal (Pons, van der Werf & Lambers 1994; Hikosaka & Terashima 1996; Evans & Poorter 2001) and plasticity in this trait thus maximizes C gain at the leaf level. That conclusion persists at the whole-plant level when all leaves are exposed to the same light conditions. However, the situation is less straightforward for whole-plant C gain in light gradients in canopies. Evans (1993b) calculated that in a dense canopy more partitioning to light harvesting (as found in lower leaves), applied to all leaves, reduced C gain substantially, but that the reduction was only a few per cent when all leaves had an upper-leaf type of partitioning. However, no comparisons were made between crowded and non-crowded conditions. Moreover, Evans (1993b) studied *Medicago sativa*, a shade-avoiding species that may have less plasticity in within-leaf partitioning compared to species from habitats with a wide range of light availability (Murchie & Horton 1997).

The question addressed here is: what is the adaptive significance of plasticity in response to canopy density of between-leaf and within-leaf partitioning of photosynthetic resources? Whole-plant C gain at constant whole-plant leaf N was used as a fitness measure. *Lysimachia vulgaris* L. plants were grown in open and dense stands at two nutrient availabilities. The distribution of leaf N, photosynthetic capacity and chlorophyll over the leaves of the plants were measured. We concentrated on the response of individual plants rather than on populations or canopies. *Lysimachia* has a plastic response of between-leaf partitioning to canopy density (Hirose *et al*. 1988). Part of the results of the present experiment was published earlier (Pons & Jordi 1998), where it was shown that plasticity in within-leaf partitioning was also substantial. The functional significance of the plastic response in between- and within-leaf photosynthetic resource partitioning was quantified by calculating whole-plant C gain after switching the respective distribution patterns between plants from the two canopy-density treatments in a whole-plant photosynthesis model.

Materials and methods

PLANT MATERIAL AND EXPERIMENTAL CONDITIONS

Lysimachia vulgaris is a herbaceous perennial with opposite pairs of more-or-less horizontal leaves and an erect stem. Plants were grown from rhizomes harvested from plants of the same stock that was used in a previous experiment, and the method of growing the plants was also similar (Hirose *et al*. 1988). Here we describe only the most important details. One rhizome was planted per PVC tube (diameter 4 cm; height 25 cm) filled with expanded clay granules. These tubes were placed in containers (59×35 cm^2), three for each plant density and nutrient availability. Plant densities were 440 and 40 plants m^{-2} for the high and low densities, respectively. The dense stands were surrounded by

boards covered with aluminium foil up to a height of about two-thirds of plant height. No branching occurred in the dense stands. Axillary buds of open-stand plants that started to sprout towards the end of the experiment were removed in order to avoid excessive within-plant self-shading.

The tubes with plants growing at the lower nutrient availability were kept continually in ≈10 cm depth of a complete nutrient solution containing 2 mM nitrate, among other nutrients (Hirose *et al.* 1988). A higher nutrient availability was achieved by continuously filling and draining the containers with the same nutrient solution, which flushed the tubes with the plants. The solution was renewed weekly in both treatments. The stands were grown in May and June in a glasshouse in Utrecht, the Netherlands, where day temperatures were up to ≈25 °C and night temperatures down to ≈15 °C. Irradiance in the glasshouse was ≈50% of unobstructed daylight in diffuse light conditions. When direct sunlight reached the position of the plants, irradiance was ≈70% of that outside. The gradient of relative irradiance in the stands was measured under cloudy conditions with a line sensor, as previously described (Hirose *et al*. 1988). Two measurements were made per container. Hourly means of irradiance obtained from the nearby meteorological institute (KNMI, De Bilt) were used to calculate irradiance in the glasshouse from the above-mentioned transmission factors. Daily photosynthetically active irradiance for a representative day in the first week of July 1988, when the plants were harvested, was estimated at 17·2 mol m^2 day^{-1} at the upper canopy level.

PLANT HARVEST AND ANALYSIS

Three plants were harvested per container; for two of these, photosynthesis was measured before harvest. Plants were selected that covered most of the light gradient in the stands (at least 85% of maximum plant height in that canopy). Leaf area, fresh and dry mass, and total N concentration were determined on all leaf pairs. The latter was measured after Kjeldahl digestion for organic N (Pons *et al.* 1993). Chlorophyll was measured only in leaves on which photosynthesis was also measured. It was extracted in 80% acetone and calculated according to Lichtenthaler & Wellburn (1983). As there were no significant differences between plants from the three containers within each treatment, the experiment was further treated as fully randomized.

Irradiance-response curves of net photosynthesis were measured on two attached leaves per plant at different heights along the stem. An open system for gas-exchange measurement was used as described by Schieving *et al.* (1992). Larger leaf chambers were used with a window of 69 × 67 mm^2 (Pons & Welschen 2002) that accommodated most of the area of the leaves. Leaf temperature was kept at 20 °C, vapour pressure difference at ≈0·6 kPa and CO$_2$ partial pressure in the chambers at ≈34 Pa. The differences in partial pressure of

CO$_2$ and H$_2$O between inlet and outlet air were measured with differential IRGAs (MK3, ADC, Hoddeson, UK). Net photosynthesis was calculated according to von Caemmerer & Farquhar (1981) and expressed per unit leaf area. Respiration (R_D) was measured after 20 min in the dark.

CALCULATION OF WHOLE-PLANT CARBON GAIN

Instantaneous rates of gross photosynthesis (A_g) at different photon flux densities (PFD) were calculated by adding dark respiration (R_D) to net photosynthetic rates. The data were subsequently fitted to a non-rectangular hyperbola describing the response of A_g to PFD (Hirose & Werger 1987a; Pons *et al*. 1989; Kull 2002) (Table 1). Three parameters were derived from this procedure: the asymptote of the hyperbola (A_g at infinitely high PFD, A_{max}); the initial slope at infinitely low PFD (apparent quantum yield, Φ); and the curvature of the transition of the light-limited to the light-saturated part of the relationship (Θ). A_{max} was subsequently fitted to N per unit leaf area (N_{LA}) using linear regression. R_D was expressed as a fraction of A_{max}. The parameters Φ and Θ were fitted to chlorophyll per unit leaf area using a hyperbolic and a linear equation, respectively. The equations used for the fitting procedures are shown in Table 1.

Data on the distribution of relative irradiance in the stands were logarithmically transformed and regressed on height in the canopy using a fourth-degree polynomial. Relative PFD incident on the leaves could be derived from these irradiance profiles, because their orientation was predominantly horizontal. N_{LA} was regressed on relative PFD after logarithmic transformation of both parameters (Table 1). A_{max} per unit chlorophyll (A_{max}/chl) was also regressed on relative PFD after logarithmic transformation of the latter (Table 1). These relationships were used to derive smoothed height profiles of, respectively, A_{max} as being representative of partitioning of resources for photosynthetic capacity between leaves, and A_{max}/chl as representative of partitioning within leaves.

For the calculation of whole-plant C gain in the stands, plants were placed in discrete categories of height relative to the highest plant in the canopy, and the mean distribution of leaf area per plant was calculated. The distributions of N_{LA} and A_{max}/chl, respectively, the between-leaf and within-leaf partitioning parameters, were calculated from the relative PFD in the middle of each layer using the N_{LA}–PFD and A_{max}/chl–PFD relationships. N_{LA} was subsequently used to calculate A_{max}, and R_D was calculated from A_{max} in the same way as discussed above (Table 1). Chlorophyll was calculated from A_{max} and A_{max}/chl, and Φ and Θ were calculated from chlorophyll. All parameters of the A_g–PFD curve were now defined for each layer. The PFD data used for the calculation of daily whole-plant photosynthesis were obtained from the hourly

Table 1. Equations used in calculations of whole-plant photosynthesis and parameter values of regressions derived from the experimental treatments: low and high nutrient availability (LN, HN), and open and dense stands (O, D)

Equations	Treatment	Coefficient b	Constant a	Asymptote m	r^2
Photosynthesis–PFD relationship					
$A_G = \{\Phi I + A_m - \sqrt{[(\Phi I + A_m)^2 - 4\Phi\Theta I A_m]}\}/2\Theta$					
Parameters A–PFD relationship					
$A_m = bN_{LA} + a$	all	0·254	−3·92		0·895
$R_D = bA_m$	all	0·0401			0·840
$\Theta = b\mathrm{Chl} + a$	all	$-6\cdot19E^{-4}$	1·004		0·376
$\Phi = m[1 - \exp(-b\mathrm{Chl})]$	all	$8\cdot86E^{-3}$		0·0651	0·458
N_{LA}–PFD relationships					
$\ln(N_{LA}) = b\ln(I_r) + a$	LN O	0·773	4·17		0·413
	LN D	0·205	3·97		0·813
	HN O	0·423	4·34		0·343
	HN D	0·214	4·37		0·905
A_{max}/chl–PFD relationships					
$\ln(A_C) = b\ln(I_r) + a$	LN O	0·330	3·73		0·143
$\ln(A_C) = m(1 - \exp\{-b[\ln(I_r) - a]\})$	HN D	0·480	3·84		0·465
	LN O	0·248	−7·44	4·56	0·973
	HN D	0·487	−6·94	3·99	0·920

A_g, gross photosynthetic rate (net rate + R_D), µmol m^{-2} s^{-1}; A_{max} (A_m), light-saturated rate of gross photosynthesis at ambient CO_2, µmol m^{-2} s^{-1}; A_{max}/chl (A_C), A_{max} per unit chlorophyll, mmol mol^{-1} s^{-1}; Chl, chlorophyll, normally expressed per unit leaf area, µmol m^{-2}; I, photosynthetic irradiance (PFD), µmol m^{-2} s^{-1}; I_r, I in a canopy relative to that above the canopy; PFD, photon flux density of photosynthetically active radiation, µmol m^{-2} s^{-1}; N_{LA}, leaf nitrogen per unit leaf area, mmol m^{-2}; R_D, rate of respiration in the dark, µmol m^{-2} s^{-1}; Φ, apparent quantum yield, mol mol^{-1}; Θ, curvature parameter of the photosynthesis–PFD relationship.

records of mean irradiance for a representative day as mentioned above, and the profiles of relative PFD in the stands. Light was treated as diffuse only as in the canopy photosynthesis model of Hirose & Werger (1987a). This was valid because including the small direct light component in the glasshouse in the calculations did not alter the results qualitatively.

ESTIMATING THE FUNCTIONAL SIGNIFICANCE OF PLASTICITY IN BETWEEN- AND WITHIN-LEAF PARTITIONING

Sensitivity analyses were carried out to evaluate the functional significance of plasticity in the two partitioning parameters for C gain in stands of different densities. This was done by switching the distribution of N_{LA} and A_{max}/chl between open and dense stands, and recalculating daily C gain. The distribution of leaf area and total leaf N of the plant were kept constant. Thus we are able to compare the photosynthetic performance of a plant that plastically adjusts its partitioning to a dense-stand condition with that of a hypothetical alternative plant that rigidly maintains an open-stand distribution pattern, and *vice versa*. For evaluating between-leaf resource partitioning, the distribution of N_{LA} over the layers was switched between the two stands, while keeping the chlorophyll content of the leaves constant. The switch thus pertains to resources associated with photosynthetic capacity only. In order to correct for the different total leaf N per plant in open and dense stands, N_{LA} in all layers was multiplied by a fixed factor after the switch. A_{max} and

R_D were derived from the switched distributions of N_{LA} and daily whole-plant C gain was recalculated.

The switch of the distribution of within-leaf resource partitioning between stands was carried out while keeping the distribution of photosynthetic N among leaves constant. Leaf N associated with the two main photosynthetic functions in the leaf, photosynthetic capacity and light harvesting, that together form total photosynthetic N, were calculated from A_{max} and chlorophyll and their respective N costs. For the specific N cost of light harvesting, 41 mol N mol^{-1} chlorophyll was used, as estimated by Evans & Seemann (1989). The specific N cost of photosynthetic capacity was derived from the slope of the A_{max}–N_{LA} relationship [3·93 mol N (mmol CO_2 m^{-2} s^{-1})$^{-1}$]. After the switch of the distribution of A_{max}/chl, the distributions of A_{max} and chlorophyll were recalculated from the same specific N costs of both photosynthetic functions. Daily whole-plant C gain was now recalculated as described above.

Results and discussion

RESPONSES TO CANOPY DENSITY

Competition for light was substantial at the high stand density of the two nutrient treatments, as evident from the reduced above-ground biomass (Table 2) and the strong light extinction in the dense stands (Fig. 1). The longer internodes and smaller fraction of shoot mass in leaves of dense-stand plants (Table 2) shows also that *L. vulgaris* displayed a pronounced shade-avoidance syndrome (Smith & Whitelam 1997). The plants grown

Table 2. Plant parameters for nutrient (N) and plant density (D) treatments (means ± SE; *n* = 9): total leaf area per plant, leaf mass per unit leaf area (LMA), total leaf nitrogen per plant, number of leaf pairs, dry mass of shoot, leaf mass ratio (LMR) of shoot, mean height of plants sampled, and height of tallest plant used as a reference to calculate relative height

Parameter	Low nutrients		High nutrients		ANOVA		
	Open stand	Dense stand	Open stand	Dense stand	N	D	N × D
Leaf area (cm^2)	247 ± 19	162 ± 6	458 ± 23	338 ± 22	***	***	ns
LMA (g m^{-2})	24·5 ± 0·7	14·2 ± 0·5	24·9 ± 0·7	15·5 ± 0·4	ns	***	ns
Leaf N (mmol)	1·30 ± 0·11	0·53 ± 0·02	3·01 ± 0·09	1·52 ± 0·11	***	***	***
Number of leaf pairs	7·6 ± 0·3	6·7 ± 0·3	8·2 ± 0·4	7·3 ± 0·2	*	**	ns
Shoot dry mass (mg)	946 ± 70	428 ± 23	1809 ± 71	949 ± 58	***	***	**
LMR shoot	0·66 ± 0·01	0·57 ± 0·01	0·67 ± 0·01	0·58 ± 0·01	ns	***	ns
Mean height (cm)	33·7 ± 1·4	51·2 ± 0·9	43·0 ± 1·8	58·8 ± 0·8	***	***	ns
Max. height (cm)	38	55	50	62			

Results of two-way ANOVA shown to right (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).
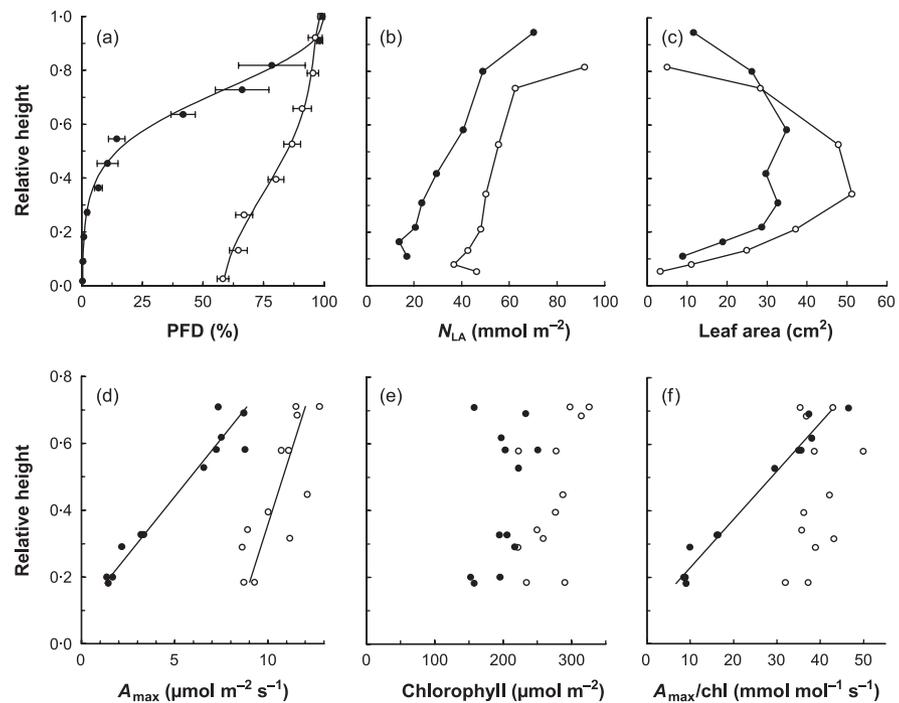

Fig. 1. Distribution of relative photon flux density (PFD) and leaf parameters over height for plants from open (○) and dense (●) stands. Data are for the low nutrient-availability treatment; for the high-nutrient treatment see Pons & Jordi (1998). Height was expressed relative to the highest plant in the stand (Table 2). (a) Relative photosynthetic PFD measured in diffuse light conditions in the two stands; (b) nitrogen per unit leaf area (N_{LA}); (c) area of all leaf pairs of one representative plant from each stand; (d) light-saturated rate of photosynthesis (A_{max}); (e) chlorophyll content; and (f) A_{max} per unit chlorophyll (A_{max}/chl) measured on two leaves from six plants from each density treatment. Smoothed PFD curves were obtained by fitting ln-transformed PFD to a fourth-degree polynomial equation. Slopes are significant where linear regression lines are depicted (d, f).

at high nutrient availability had an almost threefold greater N content in their leaves and about a twofold larger leaf area compared to the low-nutrient plants (Table 2). The gradients in N_{LA} and A_{max} over plant height were steeper for the dense stand compared with the open-stand plants (Fig. 1). This plasticity in between-leaf resource partitioning was evident in both nutrient treatments (Figs 1 and 2; Pons & Jordi 1998). Similar plasticity was found for such diverse growth forms as *Syngonium podophyllum* Ackerly 1992), *Carex acutiformis* (Schieving *et al.* 1992), *Ficus benjamina*

(Pons & Jordi 1998) and *Xanthium canadense* (Anten *et al.* 1998), and also in experimental irradiance gradients (Pons *et al.* 1993; Hikosaka *et al.* 1994; Pons & Pearcy 1994; Pons & Jordi 1998). However, separate age effects on leaf N gradients were identified in open-stand plants of *Lysimachia* (Hirose *et al.* 1988) and *X. canadense* (Anten *et al.* 1998). Mooney *et al.* (1981) identified leaf age as the principal factor causing canopy gradients in A_{max} in early successional annuals, which suggests that these plants were not plastic in their between-leaf resource partitioning.
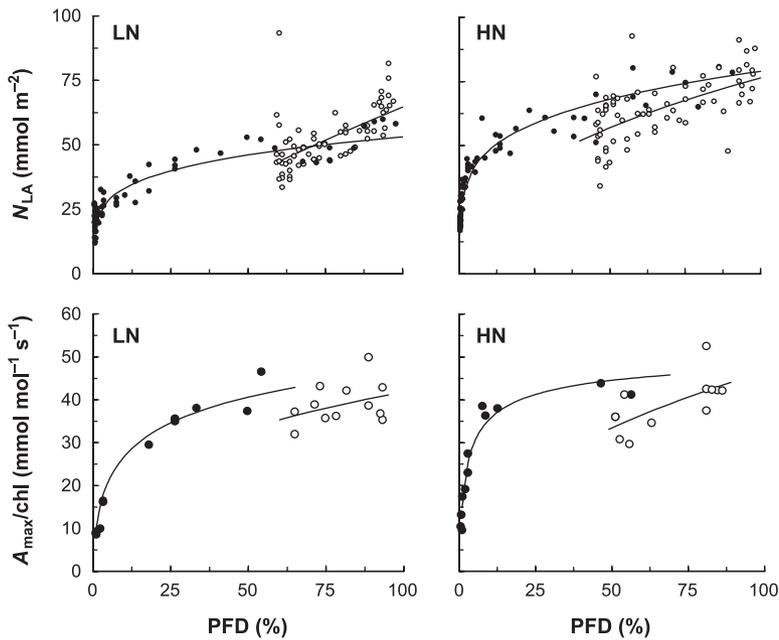
Fig. 2. Nitrogen per unit leaf area (N_{LA}) and light-saturated rate of photosynthesis per unit chlorophyll (A_{max}/chl) plotted against relative photosynthetic photon flux density (PFD) for plants from open (○) and dense (●) stands, and low (LN) and high (HN) nutrient-availability treatments. These relationships were used to describe the distribution of these parameters over plant height and switch them between stand densities. N_{LA} data are for all leaf pairs of nine plants per stand except the youngest growing top leaves. A_{max}/chl data are for two leaves from six plants from each stand. Equations and parameter values of the fitted curves are given in Table 1.
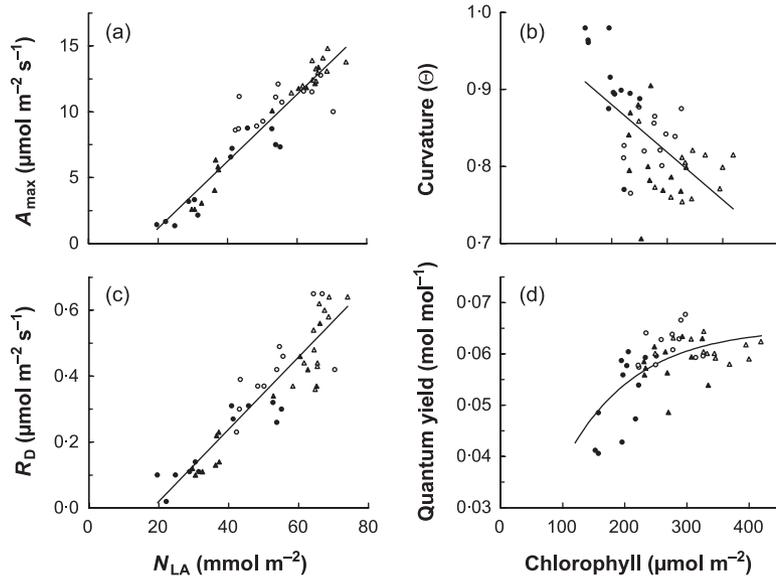

Fig. 3. Relationships with leaf nitrogen and chlorophyll of the parameters of the rectangular hyperbolic equation describing the irradiance response of photosynthesis. The light-saturated rate of (a) gross photosynthesis (A_{max}) and (c) dark respiration (R_D) were plotted against N per unit leaf area (N_{LA}). The (b) curvature parameter (Θ) and (d) apparent quantum yield (Φ) were plotted against chlorophyll content. For equations and parameter values see Table 1. Symbols: low nutrients (●, ○); high nutrients (△, ▲); open stands (○, △); dense stands (●, ▲).

ated with a low chlorophyll *a/b* ratio (data not shown; for the high-nutrient treatment see Pons & Jordi (1998). These data indicate that *Lysimachia* also exhibits strong plasticity in partitioning between photosynthetic capacity and light harvesting within leaves. This form of plasticity is found in most plants when grown at different light availabilities (Murchie & Horton 1997) and is reflected in chloroplast structure (Anderson *et al.* 1995). A gradient in A_{max}/chl is found not only between leaves in a canopy, but also between chloroplasts in the cross-section of horizontal leaves where a light gradient exists within the leaf (Terashima & Inoue 1985; Evans & Vogelmann 2003). This indicates that chloroplasts acclimate to the local light environment, which is the basis of the within-leaf partitioning of photosynthetic resources.

Open-stand plants and top leaves of dense-stand plants had a similar A_{max}/chl at the two nutrient-availability treatments. However, the reduction in A_{max}/chl with decreasing light availability in the dense stands was less strong at high compared with low nutrient availability (Fig. 2). An explanation for this difference could be that stand development at high nutrient availability was faster than at low nutrient availability, resulting in a faster reduction in irradiance in the stand, particularly at medium heights. The response time of the partitioning of resources between capacity and light harvesting on a sudden change in light gradient was around 12 days in *Glycine max* (Pons & Pearcy 1994). Hence the A_{max}/chl–PFD relationship in the dense stands (Fig. 2) probably does not reflect steady-state conditions. A_{max}/chl in the faster-growing high-nutrient plants may thus lag further behind the development of the light gradient than in the slower-growing low-nutrient plants.

Other relationships used in the model calculations are those pertaining to the light-response parameters (Fig. 3). Parameter values are shown in Table 1. The A_{max}–N_{LA} relationship was linear, with similar parameter values to those found in an earlier experiment (Pons *et al.* 1989). Higher A_{max} values, although still not very high, were now included in the range, but no evidence was found for curvilinearity as reported in other studies (Evans 1989a; Hikosaka & Terashima 1995). The relationship of dark respiration (R_D) with N_{LA} was also linear (Fig. 3, $r^2 = 0.81$) with a similar N_{LA} intercept. Hence there was a constant ratio of R_D to A_{max} across all treatments, which was used in the calculations (Table 1). The curvature factor (Θ) decreased with increasing chlorophyll as found previously (Leverenz 1987), and apparent quantum yield (Φ) increased more or less hyperbolically, as expected from a similar increase of absorptance with chlorophyll (Gabrielsen 1948). In statistical terms, chlorophyll was also better at explaining variations in Θ and Φ than was N_{LA}, which was used previously (Pons *et al.* 1989; Schieving *et al.* 1992).

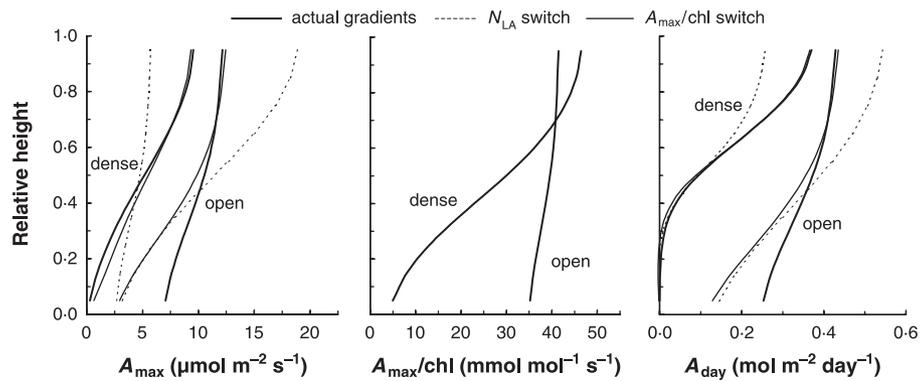Canopy profiles of photosynthetic activity (Fig. 4) were calculated from the profiles of A_{max}, chlorophyll

Chlorophyll changed little with height at both stand densities (Fig. 1), thus the gradient in A_{max}/chl was similar to the A_{max} gradient (Figs 1 and 2). A low A_{max}/chl in the lower leaves of dense-stand plants was associ-

Fig. 4. Gradients over plant height of (a) photosynthetic capacity (A_{max}) and (b) A_{max} per unit chlorophyll as used in the calculations, and (c) calculated daily net photosynthetic activity (A_{day}). Thick lines are based on measured gradients, stippled lines are the result of a switch of N_{LA} gradients at constant whole-plant leaf nitrogen between plants of the two stand-density treatments; thin continuous lines are the result of the switch of A_{max}/chl at constant photosynthetic N per leaf area. Data are for the low nutrient-availability treatment only.

Table 3. Effect of plasticity in between-leaf and within-leaf partitioning of photosynthetic resources on carbon gain for the two nutrient-availability and plant-density treatments

Parameter	Low nutrients		High nutrients	
	Open Stand	Dense Stand	Open Stand	Dense Stand
Carbon gain of whole plant				
Net C gain (mmol day^{-1})	8·45	1·58	17·11	3·81
Between-leaf partitioning:				
switch of N_{LA} between densities (%)	−2·6	−19·6	−0·9	−18·8
Within-leaf partitioning:				
switch of A_{max}/chl between densities (%)	−12·3	−5·7	−11·9	−3·7
Carbon gain of lower half of plant				
Relative irradiance (decreasing from – to)	0·82–0·59	0·10–0·01	0·78–0·46	0·04–0·01
Net C gain (mmol day^{-1})	5·11	0·25	10·97	0·07
switch of A_{max}/chl between densities (%)	−19·8	−30·5	−17·3	−164·4
Gross photosynthesis (mmol day^{-1})	5·61	0·65	12·29	0·32
switch of A_{max}/chl between densities (%)	−20·3	−15·9	−17·9	−13·7

Net whole-plant carbon gain was calculated for plant parameters as found in the treatments, and recalculated after the distribution of nitrogen per unit leaf area (N_{LA}), and thus photosynthetic capacity (A_{max}), was switched between plants from the two-canopy density treatments within one nutrient treatment keeping whole-plant leaf N constant (see text). A switch was also carried out for the distribution of photosynthetic capacity per unit chlorophyll (A_{max}/chl) at constant photosynthetic N per unit leaf area. Net and gross C gain and the effect of a switch of A_{max}/chl were also calculated for the lower half of the plants. Differences from the original values are expressed as percentages.

and PFD. Results for the low-nutrient treatment only are shown because the high-nutrient plants had similar profiles, but at about a twofold higher whole-plant C gain at both stand densities (Table 3). This was largely a result of their larger leaf area and, to a lesser extent, their higher A_{max}. Dense-stand plants were calculated to have about a fivefold smaller daily C gain than open-stand plants (Table 3). This had several causes. A_{max} was smaller in the top leaves (Fig. 1), which reduced photosynthetic performance at the prevailing high PFDs. Bottom leaves of dense-stand plants were much more strongly shaded, which resulted in a considerably lower photosynthetic activity. Whole-plant C gain was further reduced by the smaller leaf area per plant.

PLASTICITY IN BETWEEN-LEAF PHOTOSYNTHETIC RESOURCE PARTITIONING

The sensitivity of whole-plant C gain was investigated for a switch between stand densities of the distribution of N, and thus A_{max}, over the leaf area of the plants as a measure of the functional significance of plasticity in between leaf N allocation. Carbon gain of dense-stand plants at low nutrient availability was reduced substantially (19·6%) when given the less steep N_{LA} gradient of open-stand plants (Table 3). However, that difference was only 2·6% when open-stand plants were given the N_{LA} gradient of dense-stand plants. The reason for the difference in sensitivity of whole-plant C gain to the switch is shown in Fig. 4. An increase in A_{max} of top

leaves at the expense of bottom leaves of open-stand plants increased their photosynthetic activity, which almost fully compensated for the lower photosynthetic activity due to the smaller A_{max} of the bottom leaves. By contrast, in the dense-stand plants an increase in A_{max} of bottom leaves did not increase photosynthetic activity due to low light availability, whereas the resulting A_{max} of top leaves reduced their photosynthetic activity substantially. The importance for whole-plant C gain of the between-leaf photosynthetic resource partitioning in dense stands is well established (Field 1983; Hirose & Werger 1987b; Schieving *et al.* 1992; Grindlay 1997; Anten *et al.* 2000; Kull 2002) (Table 3). Field (1983) and Schieving *et al.* (1992) also mentioned that the between-leaf distribution is less relevant when dealing with more open stands. The almost equal C gain of open-stand plants at contrasting patterns of between-leaf resource partitioning (Table 3) suggests that plasticity in this trait has little adaptive value, because a constitutive dense canopy-type distribution would give an almost equal C gain in an open canopy.

How, then, can we understand plasticity in between-leaf partitioning from a functional perspective? The switch at high nutrient availability of the N_{LA} gradients between stands resulted in N_{LA} values of top leaves that were outside the range of N_{LA} and A_{max} for *Lysimachia* in our experiment. A_{max} of top leaves would be 30 µmol m^{-2} s^{-1} in the open stand, a value never found for this species. This is likely to be outside the linear range of the N_{LA}–A_{max} relationship. It is likely that a given mesophyll structure sets an upper limit to A_{max} (Oguchi, Hikosaka & Hirose 2003). Plants growing at high nutrient availability are presumably close to that limit in leaves exposed to high PFD. A higher whole-plant C gain in open-canopy plants can then be achieved only by also keeping A_{max} high in bottom leaves. In the dense stand, on the other hand, there are advantages of a smaller capacity in bottom leaves at high nutrient availability. Redundancy of resources in shaded bottom leaves may not be detrimental for photosynthetic rates, but is likely to generate costs for resource uptake and assimilation, maintenance of the large photosynthetic apparatus, and vulnerability to herbivores and parasites (Stockhoff 1994). Attractiveness for herbivores of high-N_{LA} top leaves in open-canopy plants may also have selected against a constitutive N_{LA} gradient and for plasticity under high nutrient conditions. Plants growing at low nutrient availability are less restricted by an upper limit to A_{max}. Hence a constitutive dense canopy-type A_{max} gradient in open-stand plants would not impair their C gain. This predicts that plasticity in between-leaf partitioning in response to varying canopy density might be greater at high than at low nutrient availability. There was some evidence for a steeper N_{LA}–PFD relationship for open-stand plants in the low- compared with the high-nutrient treatment (Fig. 2). A more constitutive leaf N gradient independent of a light gradient was demonstrated by Hikosaka *et al.* (1994) for the annual vine *Ipomoea tricolor* at low

nutrient availability, whereas plasticity was much larger at high nutrient availability; *G. max* grown in an experimental irradiance gradient also showed such a tendency (Pons & Pearcy 1994). Low N availability stimulates reallocation of leaf N from older leaves in many species, which results in a leaf N gradient independent of an irradiance gradient. This is likely to be most pronounced in inherently fast-growing annual dicots that are characterized by high leaf turnover rates (Westoby *et al.* 2002), resulting in a constitutive leaf N gradient in these plants at low nutrient availability.

PLASTICITY IN WITHIN-LEAF RESOURCE PARTITIONING

The next step was a switch of the distribution over the plant of within-leaf partitioning between N involved in photosynthetic capacity and N involved in light harvesting, to estimate the functional significance of plasticity in within-leaf N allocation. As the chlorophyll content of the leaves was relatively constant (Fig. 1) and thus leaf N associated with light harvesting was similar for all leaves, it was assumed that all changes in N_{LA} were associated with changes in A_{max}. The slope of the A_{max}–N_{LA} relationship thus reflected the N costs of A_{max}. These costs include not only the N costs of proteins that make up photosynthetic capacity, but also those of associated processes such as synthesis and maintenance, and assimilate processing and transport. The value of 3·93 mol N (mmol CO_2 m^{-2} s^{-1})$^{-1}$ (the inverse of 0·254 in Table 1) exceeds the 3·2 estimated for direct N costs of A_{max} for a number of *Poa* species that were also measured at 20 °C (Westbeek *et al.* 1999).

When the distribution of A_{max}/chl was switched between plants from the two density treatments, while keeping photosynthetic N constant, daily C gain was reduced more in open-stand plants (average 12·1%) than in dense-stand plants (average 4·7%) (Table 3). The results are similar for the low- and high-nutrient treatments, although the reductions were smaller in the latter. This is the opposite of what was found for between-leaf partitioning, where the switch was most detrimental for dense-stand plants. The reason for the smaller C gain of open-stand plants after the switch was that the decrease of A_{max}/chl in their bottom leaves decreased A_{max} (Fig. 4). This substantially decreased photosynthetic activity in these leaves and in the whole plant. The higher A_{max}/chl at lower and particularly intermediate heights of dense-stand plants after the switch reduced the chlorophyll content, and thus quantum yield, which reduced photosynthetic performance in low-light conditions (Fig. 1). However, that was less important for whole-plant C gain as most photosynthetic activity was concentrated in the upper leaves, where there was little change as a result of the switch (Fig. 4). These results, and those of Evans (1993b), suggest that plasticity of within-leaf partitioning is of limited importance for C gain of plants in

dense stands, as a constitutive open-canopy partitioning would not be a big disadvantage. Nevertheless, the few per cent gained by plasticity in within-leaf partitioning may still be important in competing plants.

The conclusion that dense-stand plants did not benefit much from plasticity in within-leaf partitioning applies to plants that have some leaves in high-light conditions where most C gain occurs. However, when the switch between the open- and dense-stand distributions of A_{max}/chl was done only for the lower half of the canopy, daily gross photosynthesis was reduced by 15% in dense-stand plants (Table 3). The advantage of an adjusted A_{max}/chl was larger when net C gain was used as the basis of comparison, because photosynthetic rates were near the compensation point, particularly in the high-nutrient plants. This means that subordinate plants that have all their leaves in shade gain substantially from plasticity in within-leaf partitioning. The acclimation of chloroplasts to the local irradiance in dense stands, as found here for *Lysimachia*, is apparently less important for C gain by a plant that has leaves across the whole light gradient. This form of plasticity has its largest adaptive value for the adjustment of photosynthetic capacity per unit chlorophyll to the more uniform whole-plant shade under leaf canopies.

Conclusions

Plasticity in partitioning of photosynthetic resources between leaves of plants growing in stands of different densities was not very important for whole-plant C gain. This was concluded from the low sensitivity of C gain in open-stand plants for between-leaf partitioning of photosynthetic capacity. That plants exhibit this form of plasticity, particularly at high nutrient availability, is probably associated with the fact that A_{max} is constrained. Plasticity in within-leaf partitioning of resources between capacity and light harvesting was also not very important for C gain, in this case for dense-stand plants with some well illuminated leaves at the top of the canopy. However, calculations showed that plasticity is important for subordinate plants that have all their leaves in shade.

Acknowledgements

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