

Increase in Leaf Mass Per Area Benefits Plant Growth at Elevated CO₂ Concentration

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An increase in leaf mass per area (M_{LA}) of plants grown at elevated [CO₂] is often accompanied by accumulation of non-structural carbohydrates, and has been considered to be a response resulting from source–sink imbalance. We hypothesized that the increase in M_{LA} benefits plants by increasing the net assimilation rate through maintaining a high leaf nitrogen content per area (N_{LA}). To test this hypothesis, *Polygonum cuspidatum* was grown at ambient (370 $\mu\text{mol mol}^{-1}$) and elevated (700 $\mu\text{mol mol}^{-1}$) [CO₂] with three levels of N supply. Elevated [CO₂] significantly increased M_{LA} with smaller effects on N_{LA} and leaf mass ratio (f_{LM}). The effect of change in M_{LA} on plant growth was investigated by the sensitivity analysis: M_{LA} values observed at ambient and elevated [CO₂] were substituted into a steady-state growth model to calculate the relative growth rate (R). At ambient [CO₂], substitution of a high M_{LA} (observed at elevated [CO₂]) did not increase R , compared with R for a low M_{LA} (observed at ambient [CO₂]), whereas at elevated [CO₂] the high M_{LA} always increased R compared with R at the low M_{LA} . These results suggest that the increase in M_{LA} contributes to growth enhancement under elevated [CO₂]. The optimal combination of f_{LM} and M_{LA} to maximize R was determined for different [CO₂] and N availabilities. The optimal f_{LM} was nearly constant, while the optimal M_{LA} increased at elevated [CO₂], and decreased at higher N availabilities. The changes in f_{LM} of actual plants may compensate for the limited plasticity of M_{LA} . © 2003 Annals of Botany Company

Key words: Elevated [CO₂], growth model, leaf mass per area, leaf mass ratio, leaf nitrogen, optimal allocation, root : shoot ratio, sensitivity analysis.

INTRODUCTION

The effect of elevated [CO₂] on plant growth is strongly influenced by nutrient availabilities (Bazzaz, 1990; Jackson and Reynolds, 1996; Lloyd and Farquhar, 1996; Stitt and Krapp, 1999). Elevated [CO₂] stimulates growth to a lesser extent when plants are grown with a limited supply of nutrients (e.g. Pettersson *et al.*, 1993; Rogers *et al.*, 1996; Curtis and Wang, 1998; Lutze and Gifford, 1998; Yin, 2002). Elevated [CO₂] has been shown to increase shoot activity (C uptake), but not root activity (N uptake), leading to a reduction in plant N concentration (e.g. Wong, 1979; Conroy *et al.*, 1992; Norby *et al.*, 1999). Since N is an essential constituent of proteins, a decrease in the plant N concentration may lead to a reduction in plant functions, particularly in photosynthetic capacity (Field and Mooney, 1986; Luo *et al.*, 1994).

Plants respond to an alteration of N availability or C/N balance in the environment by changing their root : shoot ratio (R : S; for a review, see Wilson, 1988). Brouwer (1962) and Davidson (1969) proposed the ‘functional equilibrium’ theory, namely that R : S changes to maintain the activity ratio between the shoot and root, e.g. at low N availability R : S increases to compensate for decreased N uptake. However, this theory does not hold in the strict sense, because plant nitrogen concentration is not constant across growth conditions (Thornley, 1975; Brouwer, 1983; Hirose,

1986). Hilbert (1990) established a model for optimal allocation of biomass to the root and shoot that maximizes the relative growth rate, R (symbols used in this paper are listed in the Appendix). In his model, photosynthetic activity per unit shoot mass is a function of shoot N concentration. Allocation of more dry mass to roots increases the shoot N concentration via increased N uptake, but decreases shoot mass. The optimal allocation to roots increases with decreasing N availability, as has been observed in actual plants. It is believed that plasticity in dry matter allocation contributes to increasing fitness of plants growing in environments with changing C : N supply ratios (Hirose, 1987; Wilson, 1988).

The functional equilibrium theory predicts an increase in the R : S ratio and a reduction in leaf mass ratio, f_{LM} (the fraction of plant mass in the leaf) in plants growing at elevated [CO₂] (Wilson, 1988). However, f_{LM} in actual plants does not necessarily respond to elevated [CO₂] as expected (Stulen and den Hertog, 1993; Luo *et al.*, 1999). While there have been several reports that elevated [CO₂] decreases f_{LM} (e.g. Larigauderie *et al.*, 1988; Wilson, 1988; Bazzaz, 1990; Dippert *et al.*, 1995), other studies have shown that f_{LM} is unaffected by elevated [CO₂] (Pettersson *et al.*, 1993; Curtis and Wang, 1998; Atkin *et al.*, 1999; Norby *et al.*, 1999). The lack of an optimal response to elevated [CO₂] has been attributed to the evolutionary history of plants. Prior to the Industrial Revolution in the 19th century, atmospheric CO₂ concentrations had been stable for 200 000 years, at less than 30 Pa (Barnola *et al.*,

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1987), and plants had no opportunity to develop mechanisms that would benefit growth at elevated [CO₂] (Tissue *et al.*, 1995).

Plant growth has been analysed using relative growth rate, which is factorized as $R = E (f_{LM}/M_{LA})$, where E is net assimilation rate, and M_{LA} leaf mass per area. This equation indicates that plant growth is influenced by a structural variable M_{LA} , other than $R : S$ or f_{LM} , such that an increase in M_{LA} leads to a reduction in R , unless E and f_{LM} change simultaneously. Many studies have shown that M_{LA} consistently increases under elevated [CO₂] (Poorter *et al.*, 1996; Yin, 2002) and this increase has been primarily explained by accumulation of non-structural carbohydrates (Poorter *et al.*, 1997). Since the increase in M_{LA} leads to a reduction in R , it has been considered as a response resulting from source–sink imbalance. However, Luo *et al.* (1994) suggested a possible advantage of increasing M_{LA} under elevated [CO₂], because it would contribute to increasing leaf N content on an area basis (N_{LA}): $N_{LA} = N_{LM}M_{LA}$, where N_{LM} is leaf N concentration on a mass basis. The decrease in N_{LM} as a result of elevated [CO₂] may be compensated for by an increase in M_{LA} , to maintain a high N_{LA} (Luo *et al.*, 1994; Peterson *et al.*, 1999a). More than half the nitrogen in a leaf is in the photosynthetic apparatus (Hikosaka and Terashima, 1996), and E is an increasing function of N_{LA} (Hirose, 1984). Therefore, an increase in M_{LA} may lead to an increase in E and, consequently, in R .

In previous studies on the optimal allocation under elevated [CO₂], the effect of M_{LA} has been ignored. For example, the model of Hilbert *et al.* (1991) assumed constant M_{LA} irrespective of [CO₂] levels. Although Luo *et al.* (1994) indicated that M_{LA} was important, their model did not explain the extent to which plant growth rate changes depending on M_{LA} . On the other hand, Hirose (1987, 1988) demonstrated the importance of M_{LA} for regulating R under different N availabilities, but the implication of this at elevated [CO₂] was not considered.

The present study aims to evaluate the importance of the increase in M_{LA} on plant growth under elevated [CO₂]. *Polygonum cuspidatum* was grown with different levels of N availability at ambient and at elevated [CO₂], and growth, dry mass allocation and N concentration were determined. Incorporating the relationships between N_{LA} and E at ambient and elevated [CO₂], a steady-state growth model was constructed to predict relative growth rate in response to changes in f_{LM} and M_{LA} . Sensitivity analyses were carried out on this model to assess the effects of changes in M_{LA} on R .

MATERIALS AND METHODS

Plant material and growth conditions

A perennial herb *Polygonum cuspidatum* Sieb. et Zucc. (Japanese knotweed; syn. *Reynoutria japonica* Houttuyn) was used for the experiment. This species is distributed over a wide altitudinal range in Japan, and grows rapidly in fertile environments but shows slow but steady growth in nutrient-poor environments (Chiba and Hirose, 1993).

Seeds of *P. cuspidatum*, collected from a single clone growing on Mt Fuji, Japan, in November 1999, were sown on wet filter paper on 12 May 2000, and allowed to germinate in a growth chamber at 25 °C with light. On 17 May, they were transplanted to 4.0-l plastic pots, 16 cm in diameter, filled with washed river sand to a depth of 16 cm. Each pot contained four seedlings. Plants were grown under natural light conditions in two 2 × 2 × 2 m open-top chambers in which the CO₂ concentration was either ambient (370 μmol mol⁻¹) or elevated (700 μmol mol⁻¹). An earlier experiment had shown no significant difference between the two chambers other than in [CO₂] (Nagashima *et al.*, 2003). Air temperature, photosynthetic photon flux density (PPFD) and [CO₂] in the chamber were monitored during the experimental period. Actual [CO₂] in the chamber was 320–400 μmol mol⁻¹ for the ambient treatment, and 670–730 for the elevated [CO₂] treatment. Daily maximum and minimum air temperatures ranged from 16.8–38.3 °C and 8.0–22.0 °C, respectively. Horizontal PPFD at noon ranged from 180 (on a rainy day) to 1900 μmol m⁻² s⁻¹. Each chamber contained 36 pots, which were divided into three groups. Each group received a nutrient solution differing in its NO₃⁻ content: N1 (lowest N), N2 and N3 (highest N). The composition of N1, in mol m⁻³, was: 0.60 NO₃⁻, 1.5 SO₄²⁻, 1.33 PO₄³⁻, 0.05 BO₃³⁻, 0.20 K⁺, 0.20 Ca²⁺, 1.50 Mg²⁺, 1.43 Na⁺, 0.05 Fe(III)EDTA, 0.01 Mn²⁺, 0.01 Zn²⁺, 0.10 Cl⁻, 0.001 Cu²⁺, 0.0005 MoO₄²⁻ and 0.0002 Co²⁺. The composition of N2 and N3 was the same as that for N1, except for NO₃⁻, which was five (N2) and 25 (N3) times richer than in N1, respectively. Fifty millilitres of nutrient solution was added to pots every 5 d from 22 May. Plants were watered as needed with tap water. Pots were rotated periodically to minimize differences in growth conditions among pots.

Sampling and measurements

Three pots per [CO₂] and N treatment were sampled at 10-d intervals, on 11 and 21 June and on 1 and 11 Jul. 2000 (25, 35, 45 and 55 d after transplanting, respectively). Four plants in each pot were processed separately and there were 12 plants per treatment. Plant material was divided into the following groups: leaves (including petioles), stems and roots (including hypocotyls). Leaf area was measured using a leaf area meter (LI 3100; Li-Cor, Lincoln, NB, USA). Dry weights were determined for plant parts after drying at 70 °C for 3 d in a ventilated oven. Dried materials were ground in a Wiley mill, and their N concentrations were determined using an NC-analyzer (NC-80; Shimadzu, Kyoto, Japan).

Growth analysis and steady-state-growth model

The model of Hirose (1987, 1988) was used, after modification, in the present study. Average leaf area, dry mass and N concentration were estimated between two successive harvests: day 25 to 35, day 35 to 45 and day 45 to 55 (denoted as day 30, 40 and 50, respectively). Means of leaf area and dry mass were estimated by assuming exponential growth at each harvest interval: $\bar{X} = (X_2 - X_1) / \ln X_2 - \ln X_1$, where \bar{X} is the mean for the period between

times 1 and 2, and is derived from X_1 and X_2 , the values at times 1 and 2, respectively. N concentrations were estimated as the arithmetic mean between two successive harvests. Variables in growth analysis were also calculated as the arithmetic mean for the period between two successive harvests.

The relative growth rate (R) is the product of the net assimilation rate (E) and the leaf area ratio (F):

$$R = EF \quad (1)$$

The ratio of leaf area to whole plant dry mass is expressed as the leaf mass ratio (f_{LM}) divided by leaf mass per area (M_{LA}):

$$F = f_{LM}/M_{LA} \quad (2)$$

E is a rectangular hyperbolic function of leaf N content on an area basis (N_{LA}):

$$E = a(N_{LA} - b)/(N_{LA} - b + c) \quad (3)$$

where a , b and c are constants (Hirose, 1984). N_{LA} is a product of leaf N concentration on a mass basis (N_{LM}) and M_{LA} :

$$N_{LA} = N_{LM}M_{LA} \quad (4)$$

N_{LM} is a linear function of whole plant N concentration (N_P):

$$N_{LM} = dN_P + e \quad (5)$$

where d and e are constants (Kachi and Rorison, 1989).

The plant was considered to be composed of three organs (leaves, stems and roots), and the sum of the dry mass ratio of each organ is 1:

$$f_{LM} + f_{SM} + f_{RM} = 1 \quad (6)$$

where f_{LM} , f_{SM} , and f_{RM} are the mass fractions in the leaf, stem and root, respectively. In the present model, the f_{SM} value was fixed at 0.10, because f_{SM} was nearly constant across [CO₂] and N treatments (mean \pm s. d., 0.107 \pm 0.018). Then, eqn (6) is simplified:

$$f_{RM} = 0.9 - f_{LM} \quad (7)$$

Assuming steady-state growth (Thornley, 1972), the whole plant N concentration (N_P) is expressed as the ratio of the

rate of N absorption per plant mass ($= \sigma_N f_{RM}$) to the relative growth rate ($= Ef_{LM}/M_{LA}$):

$$N_P = \frac{\sigma_N f_{RM} M_{LA}}{Ef_{LM}} \quad (8)$$

where σ_N is the rate of net N uptake per unit root mass. σ_N is expected to increase with increasing N availability. The value of σ_N was obtained experimentally.

Both E and F , the two components of R [eqn (1)], may change in response to a change in M_{LA} . As M_{LA} increases, F decreases [eqn (2)], whereas E may increase through increases in N_P , N_{LM} and N_{LA} [eqns (3)–(5) and (8)]. Combining eqns (1)–(8) gives R as a function of three independent variables (σ_N , f_{LM} and M_{LA}) with five constants, a – e , which are determined from the experiment for each CO₂ concentration.

Statistical analyses

ANOVA was used to test for the effects of [CO₂], N supply, age and their interactions on variables of plant dry mass, R , E , F , f_{LM} , M_{LA} , σ_N , N_{LM} and N_{LA} . Variance was calculated for 12 plants per treatment ($n = 12$). For the effects of [CO₂], a t -test was also used. Effects of [CO₂] on the relationships between E and N_{LA} and between N_P and N_{LM} were tested by analysis of covariance (ANCOVA). Statistical significance was assessed using Stat-View version 5.0 (SAS Institute Inc.). Mathematica 4.0 (Wolfram Research Inc.) was used for model calculations.

RESULTS

Growth, allocation and nitrogen concentration

Elevated [CO₂] significantly increased plant dry mass, and the [CO₂] effect was larger at higher rates of N supply (Tables 1 and 2): 24.4 % (N1), 45.6 % (N2) and 91.5 % (N3). While the net assimilation rate, E , increased (by +27 % on average, although there were some exceptions: N1 on day 30, and N3 on day 50), the relative growth rate, R , decreased at elevated [CO₂] (–5.2 % on average) owing to a significant reduction in the leaf area ratio, F [–5 % on

TABLE 1. ANOVA for the effect of [CO₂], N level, plant age and their interactions on plant dry mass (M), relative growth rate (R), net assimilation rate (E), leaf area ratio (F), leaf mass ratio (f_{LM}), leaf mass per area (M_{LA}), specific absorption rate (σ_N), leaf nitrogen concentration on a dry mass basis (N_{LM}) and on an area basis (N_{LA})

	M	R	E	F	f_{LM}	M_{LA}	σ_N	N_{LM}	N_{LA}
CO ₂	115.1***	12.1***	60.7***	804.5***	24.1***	859.7***	71.2***	653.1***	7.9**
N	594.8***	151.4***	79.6***	81.6***	57.6***	92.0***	189.5***	237.2***	433.8***
Age	424.7***	209.9***	25.5***	544.2***	340.2***	153.4***	274.0***	244.8***	15.9***
CO ₂ \times N	62.2***	0.7	2.9	2.3	0.7	4.3*	4.8**	6.0**	0.9
CO ₂ \times age	24.4***	17.6***	10.5***	1.4	3.8*	7.8***	5.7**	0.8	0.8
N \times age	186.1***	78.6***	53.5***	24.3***	9.1***	16.1***	69.7***	38.5***	26.0***
CO ₂ \times N \times age	14.5***	13.6***	13.7***	0.5	1.3	3.0*	0.2	3.9**	1.8

$n = 12$.

F-values with their probabilities: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.

TABLE 2. Plant dry mass (M), relative growth rate (R), net assimilation rate (F), leaf area ratio (F_{LM}), leaf mass ratio (f_{LM}), leaf mass per area (M_{LA}), specific absorption rate (σ_N), leaf nitrogen concentration on a dry mass basis (N_{LA}) and on an area basis (N_{LA}) as influenced by [CO₂] (ambient, 370 μmol mol⁻¹; elevated, 700 μmol mol⁻¹), N treatments (N1, low; N2, medium; N3, high) and age (days after transplanting)

	N treatment	Age	M (mg)		R (d ⁻¹)		E (g m ⁻² d ⁻¹)		F (m ² g ⁻¹)		f _{LM}		M _{LA} (g m ⁻²)		σ _N (gN g ⁻¹ d ⁻¹)		N _{LM} (gN g ⁻¹)		N _{LA} (gN m ⁻²)									
			Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]	Ambi-ent [CO ₂]	Elevated [CO ₂]								
N1	30	9.6	12.9	*	0.113	0.078	***	5.46	4.84	n.s.	0.0208	0.0161	***	0.642	0.629	n.s.	30.8	39.1	***	0.0086	0.0052	***	0.0310	0.0230	***	0.91	0.86	n.s.
	40	18.8	22.6	n.s.	0.032	0.039	n.s.	1.57	2.53	***	0.0202	0.0154	***	0.637	0.607	*	31.6	39.6	***	0.0059	0.0043	n.s.	0.0277	0.0222	***	0.87	0.87	n.s.
	50	37.5	44.8	*	0.099	0.095	n.s.	6.15	7.96	***	0.0162	0.0120	***	0.537	0.519	n.s.	33.2	43.4	***	0.0051	0.0033	**	0.0278	0.0213	***	0.90	0.89	n.s.
N2	30	26.0	37.8	*	0.137	0.113	**	6.56	6.84	n.s.	0.0210	0.0168	***	0.673	0.665	n.s.	32.1	39.8	***	0.0120	0.0079	*	0.0331	0.0268	***	1.00	1.00	n.s.
	40	69.8	102.3	**	0.075	0.089	n.s.	4.79	7.49	***	0.0160	0.0120	***	0.597	0.583	n.s.	37.4	48.6	***	0.0063	0.0048	n.s.	0.0272	0.0205	***	1.01	0.99	n.s.
	50	141.8	205.6	***	0.070	0.060	*	5.43	6.77	**	0.0130	0.0089	***	0.511	0.483	*	39.5	54.4	***	0.0027	0.0017	*	0.0260	0.0188	***	1.02	1.01	n.s.
N3	30	42.2	78.9	***	0.150	0.167	*	6.61	9.38	***	0.0229	0.0178	***	0.708	0.715	n.s.	31.0	40.4	***	0.0268	0.0204	**	0.0432	0.0348	***	1.30	1.31	n.s.
	40	153.6	327.3	***	0.117	0.127	n.s.	6.08	9.29	***	0.0193	0.0138	***	0.666	0.619	***	34.5	45.1	***	0.0137	0.0096	**	0.0372	0.0260	***	1.28	1.18	***
	50	448.8	783.7	***	0.101	0.060	***	6.53	5.36	***	0.0154	0.0112	***	0.591	0.544	***	38.3	48.5	***	0.0052	0.0021	***	0.0294	0.0224	***	1.11	1.07	n.s.

Mean values are presented (n = 12). Effects of [CO₂] are tested by t-test. ***P < 0.001, **P < 0.01, *P < 0.05, n.s., not significant.

average; see eqn (1)]. Accordingly, the increase in dry mass accumulation seen at elevated $[\text{CO}_2]$ should be ascribed to growth stimulation at a stage prior to the first harvest.

The effect of elevated $[\text{CO}_2]$ on the leaf mass ratio, f_{LM} , was not consistent. Whilst no significant effect was found in five out of nine treatments (Table 2), small but significant reductions were observed at N1 on day 40, N2 on day 50, and N3 on days 40 and 50 (-3.7% on average). On the other hand, elevated $[\text{CO}_2]$ significantly increased leaf mass per area, M_{LA} , at all N levels and ages ($+29\%$ on average). Therefore, the reduction in F is attributable to the increase in M_{LA} rather than the decrease in f_{LM} [Table 2; eqn (2)].

The reduction in σ_{N} with elevated $[\text{CO}_2]$ (-34% on average; Table 2) may be ascribed to a more rapid depletion of N owing to growth enhancement at elevated $[\text{CO}_2]$ (see dry mass in Table 2). Although N_{LM} was significantly decreased by elevated $[\text{CO}_2]$ at all N levels and ages (-27% on average), N_{LA} was not affected by $[\text{CO}_2]$ except for the high-N treatment on day 40 (-8%), due to the significant increase in M_{LA} [eqn (4)].

Modelling relative growth rate

All constants in the growth model [a , b , c , d and e in eqns (3) and (5)] were determined from the experiment. The difference in regression of E as a function of N_{LA} [eqn (3), Fig. 1A] was not significant for the two $[\text{CO}_2]$ treatments ($P > 0.1$, ANCOVA). However, the effect of $[\text{CO}_2]$ on E was highly significant ($P < 0.001$, ANOVA; Table 1) with E being 27% higher at elevated $[\text{CO}_2]$ (Table 2), and correlations between N_{LA} and E were significant ($P < 0.05$). Using regression equations calculated separately for both $[\text{CO}_2]$ levels by assuming $b = 0.60$ (derived from Hirose, 1984) for both $[\text{CO}_2]$ levels, values of $a = 10.3$ and $c = 0.37$ were obtained for ambient $[\text{CO}_2]$ and $a = 21.0$ and $c = 0.86$ for elevated $[\text{CO}_2]$. Figure 1B shows the relationships between plant (N_{P}) and leaf N concentration (N_{LM}). By fitting to eqn (5), the constants d and e were determined: $d = 1.03$, $e = 0.0027$ for ambient $[\text{CO}_2]$, and $d = 1.03$, $e = 0.0012$ for elevated $[\text{CO}_2]$. The slope of the two lines did not differ significantly ($P > 0.1$, ANCOVA), but the y-intercept did ($P < 0.001$, ANCOVA).

Sensitivity analyses

To assess whether the observed increase in M_{LA} contributed to the growth enhancement under elevated $[\text{CO}_2]$, R was calculated with the model using both values of M_{LA} observed at ambient and at elevated $[\text{CO}_2]$. Because the effect of $[\text{CO}_2]$ on f_{LM} was small (Table 2), the value of f_{LM} averaged over the two $[\text{CO}_2]$ treatments in each N level and age was used for model calculations to study the effect of M_{LA} on R . For σ_{N} , values observed in each $[\text{CO}_2]$, N and age treatment were used.

Sensitivity analyses are exemplified for the data on day 30 in Table 3. At ambient $[\text{CO}_2]$, the high M_{LA} (observed at elevated $[\text{CO}_2]$) slightly increased R at N1 (4%) and N2 (0.01%), but decreased it at N3 (-8%). Similar results were obtained for days 40 and 50 (see below). Across all combinations of N levels and ages, the high M_{LA} led, on

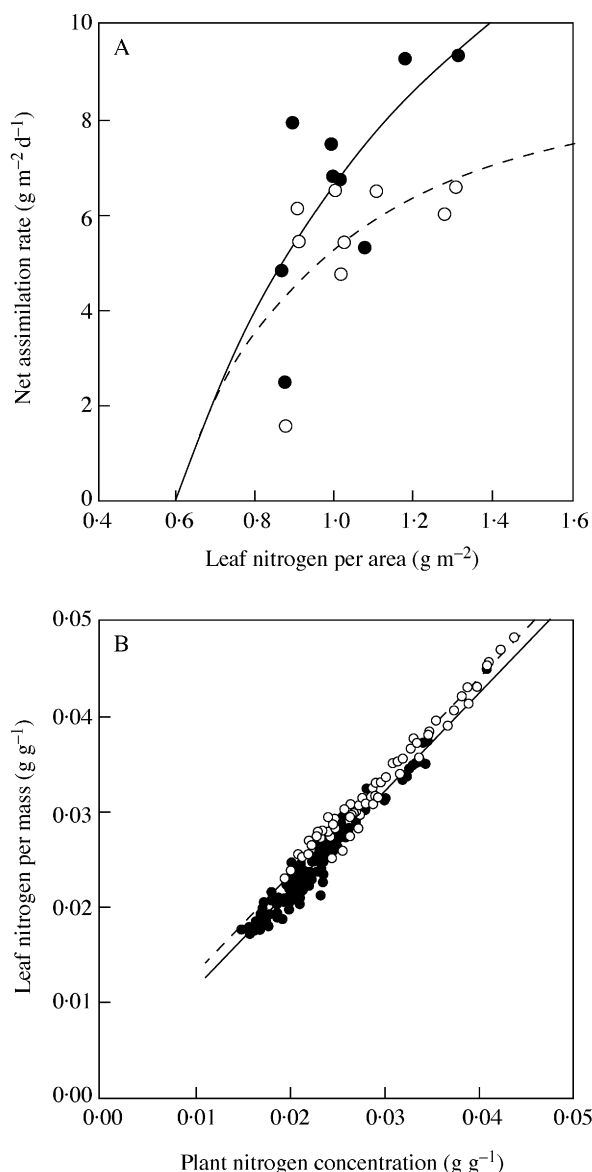


FIG. 1. Relationships between net assimilation rate (E) and leaf nitrogen content per area (N_{LA}) (A) and between leaf nitrogen per mass (N_{LM}) and plant nitrogen concentration (N_{P}) (B) in *Polygonum cuspidatum* grown at 370 (open symbols, dashed line) or 700 $\mu\text{mol mol}^{-1}$ CO_2 (closed symbols, solid line) with different rates of N supply. A, Each point is the mean for each N level and plant age. Data were fitted to a rectangular hyperbolic function [eqn (3)]: $y = 10.3(x - 0.60)/(x - 0.23)$ ($r = 0.58$), ambient $[\text{CO}_2]$; $y = 21.0(x - 0.60)/(x + 0.26)$ ($r = 0.71$), elevated $[\text{CO}_2]$. B, Each point represents an individual plant ($n = 108$). Data were fitted to linear functions [eqn (5)]: $y = 1.03x + 0.0027$ ($r = 0.98$), ambient $[\text{CO}_2]$; $y = 1.03x + 0.0012$ ($r = 0.97$), elevated $[\text{CO}_2]$.

average, to a 0.1% reduction in R at ambient $[\text{CO}_2]$. On the other hand, at elevated $[\text{CO}_2]$, the high values of M_{LA} always increased R (by 15, 11 and 6% at N1, N2 and N3, respectively), as compared with the low M_{LA} observed at ambient $[\text{CO}_2]$. Similar results were also obtained for days 40 and 50 (see below). Across all combinations of N levels and ages, the high M_{LA} led, on average, to a 13.0% increase

TABLE 3. Sensitivity analyses of the effect of leaf mass per area (M_{LA}) on leaf N content on an area basis (N_{LA}), net assimilation rate (E), leaf area ratio (F), and relative growth rate (R)

N	CO ₂	σ_N (g N g ⁻¹ d ⁻¹)	f_{LM}	M_{LA} (g m ⁻²)	N_{LA} (g N m ⁻²)	E (g m ⁻² d ⁻¹)	F (m ² g ⁻¹)	R (g g ⁻¹ d ⁻¹)
N1	Ambient	0.0086	0.64	30.8	0.87	4.34	0.0208	0.090
			0.64	39.1	1.06	5.75	0.0164	0.094
	Elevated	0.0052	0.64	30.8	0.74	2.94	0.0208	0.061
N2	Ambient	0.0120	0.64	39.1	0.82	4.28	0.0164	0.070
			0.67	32.1	0.95	5.03	0.0209	0.105
	Elevated	0.0079	0.67	39.8	1.18	6.30	0.0168	0.106
N3	Ambient	0.0268	0.67	32.1	0.79	3.83	0.0209	0.080
			0.67	39.8	0.89	5.29	0.0168	0.089
	Elevated	0.0204	0.71	31.0	1.20	6.38	0.0229	0.146
			0.71	40.4	1.68	7.67	0.0176	0.135
			0.71	31.0	0.94	5.98	0.0229	0.137
	Elevated		0.71	40.4	1.16	8.24	0.0176	0.145

Observed values of specific absorption rate (σ_N) for each [CO₂] and N-supply at day 30 were used for calculations. Leaf mass ratios (f_{LM}) were averaged across ambient and elevated CO₂ treatments.

in R at elevated [CO₂] as compared with the lower M_{LA} observed at ambient [CO₂].

Results of the sensitivity analysis are summarized in Fig. 2, in which R , calculated by substitution of the high (observed at elevated [CO₂]) M_{LA} for plants on day 30, 40 and 50 is plotted against R for the low (observed at ambient [CO₂]) M_{LA} . Open and closed circles denote plants growing at ambient and elevated [CO₂], respectively. The diagonal line indicates that R for low M_{LA} is equal to that for high M_{LA} , i.e. there is no effect of M_{LA} on R . Circles below the diagonal represent plants whose relative growth rate decreased with higher M_{LA} , while circles above the diagonal represent plants whose relative growth rate increased with higher M_{LA} . Plants growing at ambient [CO₂] were distributed along the diagonal line, indicating that some plants had higher R and others lower R with the high M_{LA} . All plants growing at elevated [CO₂] were distributed above the diagonal, indicating that they all benefited by the high M_{LA} .

Maximization of relative growth rate

Using the values of σ_N observed at ambient and at elevated [CO₂], R was calculated for various combinations of f_{LM} and M_{LA} (Fig. 3). There was an optimal combination of f_{LM} and M_{LA} that maximized R . Figure 3 shows the optimal combinations calculated for σ_N -values observed in N3 on day 30, i.e. 0.0268 and 0.0204 g N g⁻¹ d⁻¹ at ambient and elevated [CO₂]. The optimal f_{LM} was similar (around 0.46) for both [CO₂] levels, whereas the optimal M_{LA} was higher at elevated than at ambient [CO₂] (24.5 and 15.0 g m⁻², respectively).

The optimal f_{LM} and M_{LA} , and the maximized R , were plotted against σ_N together with values actually observed in the experiment (Fig. 4). The optimal f_{LM} differed little between the two [CO₂] levels, and was nearly constant across σ_N . The optimal M_{LA} , on the other hand, was higher at elevated than at ambient [CO₂] and decreased with increasing σ_N . For a given σ_N , the maximized R was higher at elevated [CO₂].

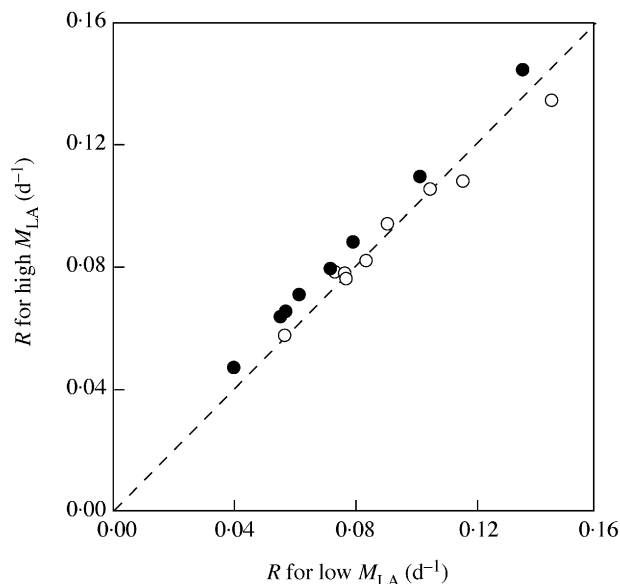


FIG. 2. Scattergram of the relative growth rate (R) calculated by substitution of a high leaf mass per area (M_{LA}) against R calculated with a low M_{LA} for plants growing at ambient (open symbols) and at elevated [CO₂] (closed symbols). High and low M_{LA} are observed values for plants grown for 30, 40 and 50 d with three N levels at elevated and ambient [CO₂], respectively. The diagonal line indicates calculated R being equal for the two M_{LA} values.

DISCUSSION

The effects of elevated [CO₂] on plants observed in the present experiment (Table 2) are mostly in accord with those reported previously (for a review, see Poorter *et al.*, 1996). [CO₂] elevation increased plant dry mass, but did not increase R . This discrepancy could be ascribed to the stimulation of growth at the stage earlier than the first harvest. Such transient stimulation of R has often been observed in other studies (e.g. Bazzaz *et al.*, 1989; Dippery *et al.*, 1995). E was increased by elevated [CO₂], while F

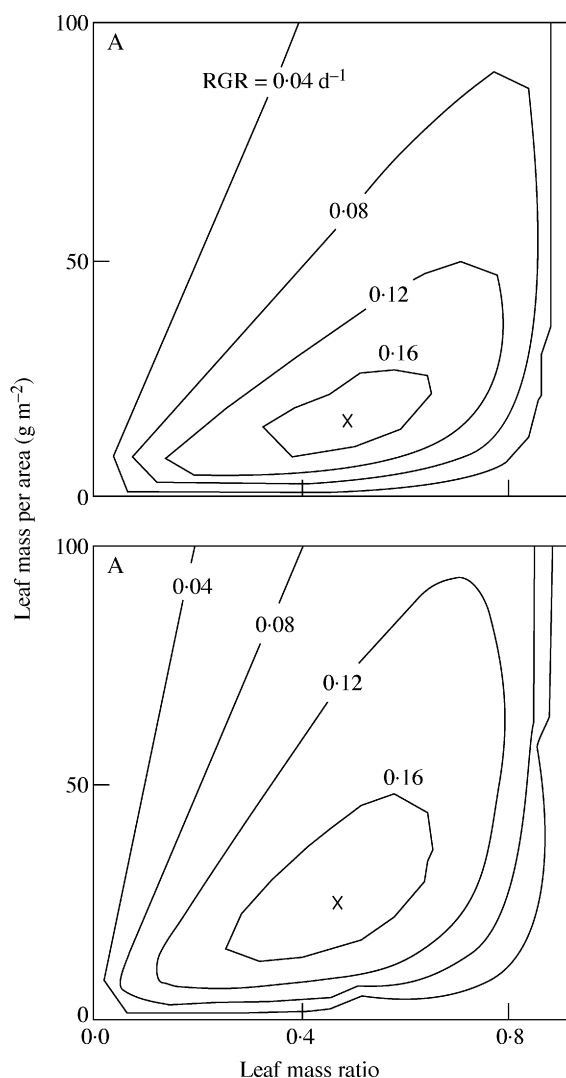


FIG. 3. Relative growth rate (R , contours) calculated by the steady-state growth model for different combinations of leaf mass ratio (f_{LM}) and leaf mass per area (M_{LA}). A, Ambient $[\text{CO}_2]$; B, elevated $[\text{CO}_2]$. The specific absorption rates (σ_{N}) observed in plants grown at high N supply (N3) on day 30 are used for the calculation: $0.0268 \text{ g N g}^{-1} \text{ d}^{-1}$, ambient $[\text{CO}_2]$; $0.0204 \text{ g N g}^{-1} \text{ d}^{-1}$, elevated $[\text{CO}_2]$. Crosses represent the coordinates that maximize R . Constants: $a = 10.3$, $b = 0.60$, $c = 0.37$, $d = 1.03$ and $e = 0.0027$, ambient $[\text{CO}_2]$; $a = 21.0$, $b = 0.60$, $c = 0.86$, $d = 1.03$ and $e = 0.0012$, elevated $[\text{CO}_2]$.

was decreased, as has been documented in a number of studies (e.g. Norby and O'Neill, 1991; Lutze and Gifford, 1998; Atkin *et al.*, 1999). Small effects of elevated $[\text{CO}_2]$ on f_{LM} are also consistent with previous studies which showed that elevated $[\text{CO}_2]$ either decreased or had no effect on f_{LM} (e.g. Stulen and den Hertog, 1993; Luo *et al.*, 1999; Norby *et al.*, 1999). On the other hand, a significant increase in M_{LA} is well documented (e.g. Arp, 1991; Luo *et al.*, 1994; Atkin *et al.*, 1999). Thus the reduction in F at elevated $[\text{CO}_2]$ was attributable mainly to the increase in M_{LA} , rather than the reduction in f_{LM} (Poorter *et al.*, 1996).

R declined faster with age at elevated $[\text{CO}_2]$ in the present experiment (Table 2). Previous studies ascribed such a

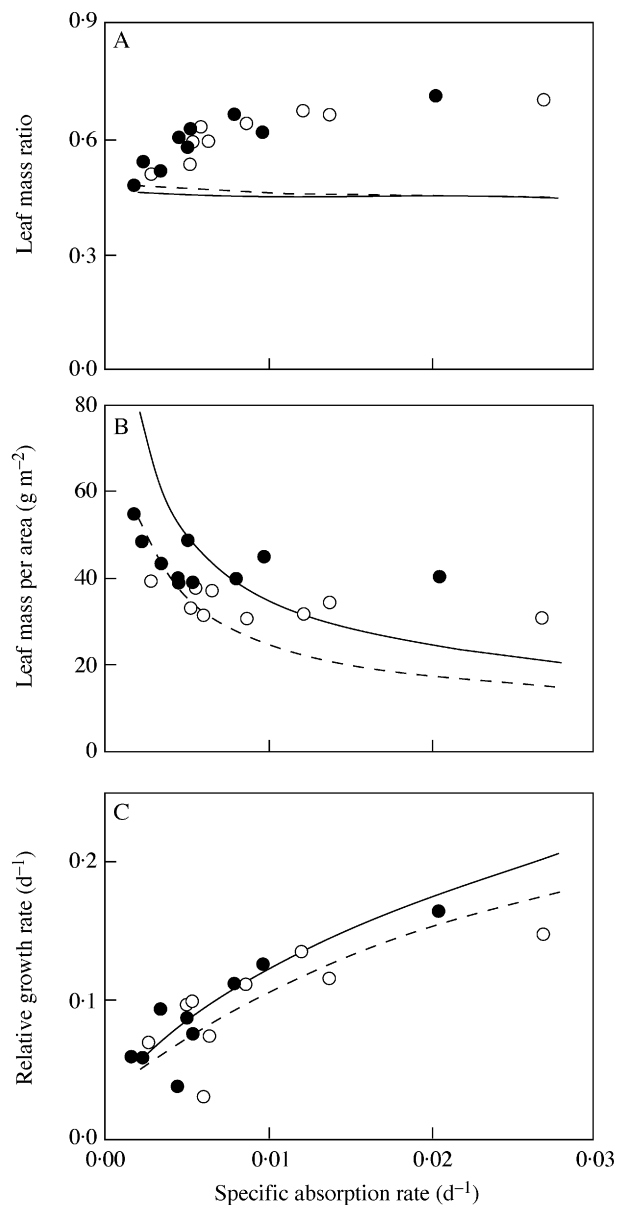


FIG. 4. The optimal leaf mass ratio (f_{LM} , A) and leaf mass per area (M_{LA} , B) that maximize the relative growth rate (R , C), plotted against specific absorption rate (σ_{N}). Lines are the theoretical optimum calculated from the model for 370 (dashed) and $700 \mu\text{mol mol}^{-1} \text{ CO}_2$ (solid). Values observed at 370 (open symbols) and $700 \mu\text{mol mol}^{-1} \text{ CO}_2$ (closed symbols). Constants are the same as in Fig. 3.

decline in growth stimulation to a reduction in E and photosynthesis by long-term exposure to elevated $[\text{CO}_2]$ (e.g. Bazzaz *et al.*, 1989; Thomas and Strain, 1991). However, our results did not show a reduction in E because N_{LA} was not decreased (Table 2; see Luo *et al.*, 1994); rather, the reduction in R should be attributed to a significant decrease in σ_{N} (Table 2). Because nutrients were not added exponentially but at a constant rate, it is likely that enhanced growth at elevated $[\text{CO}_2]$ led to a more rapid reduction in σ_{N} (Table 2), which might have decreased R via a reduction in f_{LM} (Figs 3C and 4A).

The adaptive significance of dry mass allocation has been assessed by using sensitivity analyses (Hirose 1987, 1988) and optimization models of plant growth (Hilbert, 1990; Hilbert *et al.*, 1991; Lloyd and Farquhar, 1996). However, there seems to be no theoretical study in which both f_{LM} and M_{LA} have been treated as independent variables to examine their effects on plant growth at elevated $[\text{CO}_2]$. In the present study, we tested whether the $[\text{CO}_2]$ response of actual plants benefited growth at a given $[\text{CO}_2]$ level. The present steady-state growth model described R as a function of three independent variables, σ_{N} , f_{LM} and M_{LA} , with five constants (a – e) determined for ambient and elevated $[\text{CO}_2]$. The main effect of $[\text{CO}_2]$ was exhibited in the relationship between N_{LA} and E [eqn (3) and Fig. 1A]. We assumed different equations for the relationship at both $[\text{CO}_2]$ levels, because E was significantly higher at elevated $[\text{CO}_2]$ (Tables 1 and 2) and because rates of photosynthesis per unit leaf nitrogen have been found to increase with $[\text{CO}_2]$ elevation (Hirose *et al.*, 1997; Peterson *et al.*, 1999b). E is the rate of leaf photosynthesis from which daily plant respiration is subtracted. Since $[\text{CO}_2]$ elevation increases the rate of photosynthesis with little effect on respiration (Amthor, 1997), E is expected to increase with elevated $[\text{CO}_2]$. As the rate of respiration increases with increasing protein content, E shows a more strongly curvilinear relationship with N_{LA} (Hirose, 1984) than the light-saturated rate of photosynthesis (Field and Mooney, 1986). Another seemingly less important effect of $[\text{CO}_2]$ was found in the relationship between N_{P} and N_{LM} [eqn (5) and Fig. 1B].

Sensitivity analyses have suggested that an increase in M_{LA} enhances R at elevated $[\text{CO}_2]$, while this is not the case at ambient $[\text{CO}_2]$ (Table 3; Fig. 2). This result is somewhat surprising because an increase in M_{LA} leads to a reduction in F [eqn (2)] and, consequently, in R [eqn (1)]. Increased M_{LA} may be disadvantageous because a large amount of dry mass is not used to expand leaf area. For a constant leaf N content, however, leaf area expansion is coupled with a lowering of N_{LA} (Hirose, 1984; Hikosaka and Terashima, 1995). If a plant produces a large leaf area with a limited N content, photosynthesis on a per area basis would decrease owing to a reduction in N_{LA} [eqn (3)]. The increase in M_{LA} compensated for the reduction in N_{LM} , and increased N_{LA} (Table 3), as indicated by Luo *et al.* (1994) [eqn (4)]. Thus, the increase in M_{LA} benefited the plant by increasing E [eqn (3)]; this effect was larger at elevated than at ambient $[\text{CO}_2]$ (Fig. 1A). R was not increased by an increase in M_{LA} at ambient $[\text{CO}_2]$ where the benefit of the increase in E was just offset by a simultaneous reduction in F (Table 3). On the other hand, R was increased at elevated $[\text{CO}_2]$ where an increase in M_{LA} increased E , which more than compensated for the reduction in F (Table 3). These results are in accord with those of Peterson *et al.* (1999a) who showed that whilst $[\text{CO}_2]$ elevation decreased N_{LM} in most species, it increased M_{LA} and consequently had little effect on N_{LA} . These authors demonstrated that a higher photosynthetic capacity at elevated $[\text{CO}_2]$ was dominated by the reduction in $[\text{CO}_2]$ limitation.

The increase in M_{LA} under elevated $[\text{CO}_2]$ may result from the accumulation of non-structural carbohydrates in leaves owing to source–sink imbalance and/or limitation in

the phloem-loading process (Cure *et al.*, 1991; Sims *et al.*, 1998). However, several studies have suggested that the increase in M_{LA} under elevated $[\text{CO}_2]$ was accompanied by thicker mesophyll tissues (Thomas and Harvey, 1983; Arp, 1991; Luo *et al.*, 1994). When *P. cuspidatum* was grown under conditions similar to those used in the present experiment, the starch-free leaf mass per area increased significantly (27 %) with elevated $[\text{CO}_2]$ (Y. Onoda, unpubl. res.). This may contribute to developing a large photosynthetic apparatus per unit leaf area with lower N_{LM} at elevated $[\text{CO}_2]$. Even if an increase in M_{LA} were the result of accumulation of non-structural carbohydrates (Poorter *et al.*, 1997), plants could have used these to expand their leaf area, but with a reduction in E . The present analysis suggests that the increase in M_{LA} contributed to increasing R with an increase in E at elevated $[\text{CO}_2]$ but not at ambient $[\text{CO}_2]$ (Table 3).

The present results are also informative on the issue of optimization. An optimal combination of f_{LM} and M_{LA} was calculated to maximize R . The optimal f_{LM} was found to differ only slightly between the two $[\text{CO}_2]$ levels, and was nearly constant across σ_{N} (Fig. 4A). The optimal M_{LA} , on the other hand, decreased with increasing σ_{N} , and was higher at elevated than at ambient $[\text{CO}_2]$ for a common σ_{N} (Fig. 4B). These predictions explain the $[\text{CO}_2]$ responses in actual plants, where f_{LM} was only slightly affected by $[\text{CO}_2]$ elevation, whilst M_{LA} was much increased (Table 2). However, the model predictions do not explain the actual responses to N levels (σ_{N}): the observed f_{LM} increased with increasing σ_{N} whilst the optimal f_{LM} remained constant, and the observed M_{LA} did not have such a wide range as that of the optimal M_{LA} (Fig. 4). A possible explanation for these discrepancies may lie in the constraint on plasticity in M_{LA} . M_{LA} is related to leaf thickness as well as leaf density, which changes with non-structural carbohydrate content (Poorter *et al.*, 1997). Leaf thickness and density are determined by several biological aspects other than leaf N and non-structural carbohydrates, for example, the content of defensive compounds, chlorophylls, $[\text{CO}_2]$ diffusion in the leaf, heat capacitance, transpiration, physical toughness and leaf longevity. Extremely thick leaves would be disadvantageous because the bulk resistance to $[\text{CO}_2]$ diffusion within the leaf would increase, thus lowering the efficiency of photosynthesis by reducing the CO_2 concentration in the chloroplast (Evans, 1999; Terashima *et al.*, 2001). Extremely thin leaves, on the other hand, may be fragile and may lose water easily (Gutschick, 1999). Therefore, canopy leaves that are exposed to strong light should have thick mesophyll (Terashima *et al.*, 2001). Moreover, thin leaves have a limited volume in which to accommodate chloroplasts (Oguchi *et al.*, 2003), and may also be less resistant to herbivory because the amount of defensive compounds, such as lignin and tannin, would be lower (Nabeshima *et al.*, 2001). These constraints may be involved in the optimal M_{LA} not being realized in actual plants. The plastic change in f_{LM} might have evolved as a substitute mechanism to maintain high growth rates despite the limited plasticity in M_{LA} .

Although the plants used in this experiment were not selected for in an atmosphere with high $[\text{CO}_2]$, they do

respond positively to elevated [CO₂]. How do plants sense a change in [CO₂]? The primary effect of elevated [CO₂] on a plant may be an increase in C uptake, which could also be caused by changes in other environment factors, such as high light intensity. This enhancement in C uptake changes the C/N uptake balance. To maintain a C/N balance within a plant, M_{LA} may increase when the environment favours C uptake (e.g. elevated [CO₂] and high light conditions), whilst allocation to roots may increase when the environment limits N uptake (e.g. lower nutrient availability). Even if the dry mass allocation pattern did not change as a direct response to elevated [CO₂], it could change as a secondary response through the change in C/N uptake balance.

In conclusion, the present steady-state growth model has demonstrated that the increase in leaf mass per area leads to a growth enhancement under elevated [CO₂], which has otherwise been considered to be a result of source–sink imbalance. The model predicted an optimal combination of leaf mass ratio and leaf mass per area, where the leaf mass ratio does not change in response to [CO₂] and N availability if the leaf mass per area can change plastically enough. Actual plants may have developed plasticity in their leaf mass ratio to compensate for the limited plasticity in their leaf mass per area.

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APPENDIX

TABLE A1. List of symbols used in equations

Symbols	Explanation (unit)	
E	Net assimilation rate	Rate of dry mass increase per unit leaf area (g m ⁻² d ⁻¹)
F	Leaf area ratio	Ratio of leaf area to plant mass (m ² g ⁻¹)
f_{LM}	Leaf mass ratio	Fraction of plant mass in leaf (g g ⁻¹)
f_{RM}	Root mass ratio	Fraction of plant mass in root (g g ⁻¹)
f_{SM}	Stem mass ratio	Fraction of plant mass in stem (g g ⁻¹)
M_{LA}	Leaf mass per area	Leaf mass per unit leaf area (g m ⁻²)
N_{LA}	Leaf N per area	Leaf N per unit leaf area (g N m ⁻²)
N_{LM}	Leaf N concentration	Leaf N per unit leaf mass (g N g ⁻¹)
N_P	Plant N concentration	Plant N per plant mass (g N g ⁻¹)
R	Relative growth rate	Rate of dry mass increase per unit plant mass (g g ⁻¹ d ⁻¹)
σ_N	Specific absorption rate	Rate of N uptake per unit root mass (g N g ⁻¹ d ⁻¹)
Equations		
$R = EF$		
$F = f_{LM} / M_{LA}$		
$E = a(N_{LA} - b)/(N_{LA} - b + c)$		
$N_{LA} = N_{LM}M_{LA}$		
$N_{LM} = dN_P + e$		
$f_{LM} + f_{SM} + f_{RM} = 1$		
$N_P = (\sigma_N f_{RM} M_{LA}) / (E \cdot f_{LM})$		