Annals of Botany **86**: 723–730, 2000
doi:10.1006/anbo.2000.1234, available online at http://www.idealibrary.com on IDEAL®

Compensatory Roles of Nitrogen Uptake and Photosynthetic N-use Efficiency in Determining Plant Growth Response to Elevated CO_2: Evaluation Using a Functional Balance Model

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Received: 22 January 2000 Returned for revision: 27 April 2000 Accepted: 13 June 2000 Published electronically: 10 August 2000

We used a modified functional balance (FB) model to predict growth response of *Helianthus annuus* L. to elevated CO_2. Model predictions were evaluated against measurements obtained twice during the experiment. There was a good agreement between model predictions of relative growth rate (RGR) responses to elevated CO_2 and observations, particularly at the second harvest. The model was then used to compare the relative effects of biomass allocation to roots, nitrogen (N) uptake and photosynthetic N-use efficiency (PNUE) in determining plant growth response to elevated CO_2. The model predicted that a rather substantial increase in biomass allocation to root growth had little effect on whole plant growth response to elevated CO_2, suggesting that plasticity in root allocation is relatively unimportant in determining growth response. Average N uptake rate at elevated compared to ambient CO_2 was decreased by 21–29 %. In contrast, elevated CO_2 increased PNUE by approx. 50 % due to a corresponding rise in the CO_2-saturation factor for carboxylation at elevated CO_2. The model predicted that the decreased N uptake rate at elevated CO_2 lowered RGR modestly, but this effect was counterbalanced by an increase in PNUE resulting in a positive CO_2 effect on growth. Increased PNUE may also explain why in many experiments elevated CO_2 enhances biomass accumulation despite a significant drop in tissue nitrogen concentration. The formulation of the FB model as presented here successfully predicted plant growth responses to elevated CO_2. It also proved effective in resolving which plant properties had the greatest leverage on such responses. © 2000 Annals of Botany Company

Key words: Elevated CO_2, functional balance model, *Helianthus annuus* L., N uptake, photosynthetic nitrogen use efficiency, root : shoot ratio.

INTRODUCTION

It is well established that the long-term growth response of plants to elevated CO_2 is critically dependent on availability and uptake of growth limiting nutrients (Bazzaz, 1990; Poorter, 1998; Stittm and Krapp, 1999). Therefore, models designed to predict plant growth responses to increased levels of atmospheric CO_2 need to incorporate how CO_2 affects plant capacity to acquire nutrients, particularly N. A common modelling approach to predict plant growth and allocation responses to environmental factors is based on the functional balance (FB) concept of Brouwer (1962) and Davidson (1969) which has since been refined by other researchers (e.g. Reynolds and Thornley, 1982; Gutschick, 1993; Luo *et al*., 1994; Hunt *et al*., 1998). According to this concept, growth responses to changes in the levels of environmental factors are driven by a combination of adjustments in size and/or activity of the root and shoot so as to maintain a balance between the total shoot and root activities. Thus, for example, the FB model predicts that an increase in atmospheric $[CO_2]$, which increases total carbon uptake by the shoot, should elicit root level adjustments to increase total root N uptake.

Plants possess a suite of potential mechanisms that could enhance their capacity to capture N including (*a*) greater carbon allocation to roots, and (*b*) enhanced root physiological uptake capacity for N. Both these adjustments would require additional expenditure of carbon and should potentially benefit the plant under elevated CO_2. Much of the earlier work on elevated CO_2 levels reported increased biomass allocation to the root (e.g. Oechel and Strain, 1985, also see review by Bazzaz, 1990) and qualitatively invoked the FB as an operative principle for such a shift (Wilson, 1988). However, it is increasingly evident that increased biomass allocation to roots is not a universal response to high CO_2 (reviewed in Curtis and Wang, 1998; Norby *et al*., 1999). In fact, even in cases where elevated CO_2 increases root to shoot ratio, the increase may be more related to N deficiency than to a CO_2 effect *per se* (Bazzaz, 1990; Norby *et al*., 1999). Furthermore, the few studies that have addressed the responses of root activities to high CO_2 have produced equally inconsistent results. For example, responses of specific root N uptake to CO_2 show increase, decrease or no changes (reviewed in Luo *et al*., 1999). However, such observations do not invalidate the FB approach as a predictive tool. Rather, the observed inconsistencies indicate that other compensating factors must be considered. One such a factor is PNUE (see Appendix for full list of abbreviations). The inclusion of

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PNUE as a parameter is particularly important when the FB model is used to predict growth response to elevated CO_2 because elevated CO_2 commonly increases PNUE (Drake *et al.*, 1997; Davey *et al.*, 1999; Peterson *et al.*, 1999) and because PNUE is inversely related to plant N demand. Thus, PNUE is likely to interact with, and determine the level of adjustment in, root allocation and uptake capacity in response to elevated CO_2. Therefore, a more accurate prediction of growth responses to high CO_2 may require a FB model that allows examination of the roles of root biomass allocation, specific nutrient uptake rate and PNUE collectively. Here, we use a modified FB model (Gutschick, 1993; Gutschick and Kay, 1995) which incorporates biomass allocation to root, N uptake and PNUE. The model is used to evaluate the relative contribution of root allocation, N uptake and PNUE in determining plant growth responses to elevated CO_2.

MODEL DESCRIPTION

The FB model used here to analyse plant growth response to elevated CO_2 and N is an elaboration of that derived by Gutschick (1993) and Gutschick and Kay (1995); extensive implications of the model are discussed in those manuscripts. The model equates relative growth rate (RGR) limited by carbon gain, R_{CL}, with that limited by N uptake, R_{NL}.

Carbon-limited growth rate

Relative growth rate limited by carbon gain, R_{CL}, can be expressed as:

$$R_{CL} = \dot{M}_{CL}/m_{pl} \tag{1}$$

where $\dot{M}_{CL}$ is the rate of dry mass gain, and m_{pl} is whole-plant mass ($= m_s + m_r$, the sum of shoot and root masses).

In turn, $\dot{M}_{CL}$ is related to whole-plant photosynthetic rate as:

$$\dot{M}_{CL} = \beta a_{pl}\bar{A} \tag{2}$$

where β is the efficacy of converting photosynthate to dry matter, a_{pl} is plant leaf area, and $\bar{A}$ is mean assimilation rate per area.

The terms a_{pl} and $\bar{A}$ in eqn (2) can be defined so that

$$a_{pl} = \alpha_L(m_s/m_{L,a}) \tag{3}$$

where α_L is the fraction of shoot dry mass allocated to leaves, and $m_{L,a}$ is mean leaf mass per leaf area; and mean assimilation rate ($\bar{A}$) is

$$\bar{A} = N_a p^* \tag{4}$$

where p^* is photosynthetic N-use efficiency, and N_a is the mean mass of N per leaf area, given by

$$N_a = f_{N,L} m_{L,a} \tag{5}$$

and $f_{N,L}$ is the N fraction in leaf dry mass.

Shortly, we resolve CO_2 and other physiological effects on p^*. To this point, combining eqns (2)–(5) gives:

$$\dot{M}_{CL} = \beta\alpha_L m_s f_{N,L} p^* \tag{6}$$

This gives,

$$R_{CL} = \beta\alpha_L f_{N,L}\, p^*/(1 + r) \tag{7}$$

where $r = m_r/m_s$, the root to shoot ratio.

Relative growth rate limited by N uptake

The N-limited growth rate, $\dot{M}_{NL}$, can be expressed as:

$$\dot{M}_{NL} = \dot{M}_N/f_{N,W} \tag{8}$$

where $\dot{M}_N$ is the total rate of gain in mass of N, and $f_{N,W}$ is the mass fraction of N in the whole plant. In turn, $\dot{M}_N$ is given by

$$\dot{M}_N = m_r\bar{v} \tag{9}$$

where m_r is root dry mass and $\bar{v}$ is N uptake rate per dry mass of root. Thus, from eqns (8) and (9),

$$\dot{M}_{NL} = m_r\bar{v}/f_{N,W} \tag{10}$$

and with $r = m_r/m_s$,

$$R_{NL} = r\bar{v}/[f_{N,W}(1 + r)] \tag{11}$$

In functional balance, $R_{CL} = R_{NL}$. This relation specifies $f_{N,W}$ and $f_{N,L}$ ($= \alpha_N f_{N,W}$; α_N is N-partitioning ratio to leaf, and $f_{N,L}$ is leaf N fraction) as dependent upon all other plant and environmental parameters. This yields the following expression for $f_{N,W}$:

$$f_{N,W} = \sqrt{\frac{r\bar{v}}{\beta\alpha_L\alpha_N p^*}} \tag{12}$$

Similarly, at functional balance [equating eqn (7) to eqn (11), and substituting $f_{N,L}/\alpha_N$ for $f_{N,W}$ in eqn (5)] the expression for $f_{N,L}$ is:

$$f_{N,L} = \sqrt{\frac{r\bar{v}\alpha_N}{\beta\alpha_L p^*}} \tag{13}$$

Substituting eqn (12) into either R_{NL} (or into R_{CL} and using the relation $f_{N,L} = \alpha_N f_{N,W}$) yields the RGR at functional balance, R_{FB}:

$$R_{FB} = [\sqrt{r}/(1 + r)]\sqrt{\beta\alpha_L\alpha_N p^*\bar{v}} \tag{14}$$

This expression is very similar to that in the original model, with the added resolution of N-partitioning to leaf, α_N. Now consider photosynthetic N-use efficiency, p^*. If most photosynthesis occurs at or above light-saturation, we may express assimilation in the formulation of Farquhar *et al.* (1980, and later elaborations) as:

$$A = V_{c,max}(C_i - \Gamma)/(C_i + K_{co}) \tag{15}$$

Here, C_i is leaf-internal CO_2 partial pressure, Γ is CO_2 compensation partial pressure, and K_{co} is effective Michaelis constant for CO_2 in the presence of O_2. Both Γ and K_{co} are functions only of leaf temperature, thus Γ and K_{co} have been corrected for growth temperature according to de Pury and Farquhar (1997). Taking $p^* = \underline{A}/N_a$, we may factor out the dependence on (internal) CO_2 level as:

$$p^* = [V_{c,max}/N_a][(C_i - \Gamma)/(C_i + K_{co})] \qquad (16)$$

We redefine the two terms in the brackets as $[p_o][C_{i\text{-fac}}]$; p_o is the CO_2-saturated photosynthetic N-use efficiency and $C_{i\text{-fac}}$ is the CO_2-saturation factor for carboxylation. The final expression for RGR at functional balance is:

$$R_{FB} = [\sqrt{r}/(1+r)]\sqrt{\beta \alpha_L \alpha_N p_o (C_{i\text{-fac}})\bar{v}} \qquad (17)$$

This final expression [eqn (17)] for RGR is similar to the original FB model but, as mentioned earlier, it has an added resolution since it includes α_L, and also partitions p^* into p_o and $C_{i\text{-fac}}$.

MATERIALS AND METHODS

Plant material and growth conditions

Helianthus annuus 'Teddy Bear' (seed source: Thompson & Morgan Ltd, UK) was used for the experiment reported here. The experiment was carried out in late summer–early autumn under natural light and thermal regimes. Three seeds per pot were planted directly into 2 l plastic pots filled with river-washed sand. Pots were randomly placed into 20 open-top chambers inside a greenhouse at the University of Illinois at Chicago. The sand was kept moist by watering with de-ionized water every day until seedling emergence was completed (10 d after planting, DAP). Seedlings were thinned to one per pot 11 DAP, and the treatments were started on the same day. The treatments were two CO_2 partial pressures (36 and 70 Pa) and three nitrogen levels (0·5, 2·5 and 5·0 mol m^{-3} N) supplied as NH_4NO_3. Plants were supplied with 200 ml nutrient solution every other day alternated with a similar volume of de-ionized water. The basal nutrient solution used for feeding plants contained the following macro-nutrients (mol m^{-3}): 1·2 K^+; 0·5 Mg^{2+}; 1·1 PO_4^{3-}; 0·75 Ca^{2+}; 0·5 SO_4^{2-}; 1·5 Cl^-. Micro-nutrients other than Cl^- were supplied at the following concentrations (mmol m^{-3}): 10 Fe, as ferric citrate ($FeC_6H_5O_7$); 5 BO_3^{3-}; 0·02 Co^{2+}; 0·1 Cu^{2+}; 1 Mn^{2+}; 0·5 MoO_4^{2+}; 0·1 Zn^{2+}.

The experiment was laid out as a split-plot design in ten blocks, with the CO_2 and N treatments as the main- and sub-plots, respectively. Treatments were randomly allocated both at the main- and sub-plot level.

Photosynthesis measurement

Photosynthesis was measured on the youngest fully expanded leaves twice (on days 18 and 38 after the start of the treatments) during the experiment using a portable open gas exchange system (Li-6400, Li-Cor, Inc., Lincoln, NE, USA) that was equipped with an LED light source and a CO_2 source and mixer. Measurements were made under the following conditions: 1500 µmol photons m^{-2} s^{-1} (from LED source); leaf temperature of 25°C; and 55–60 % RH. Photosynthesis measurements were made at growth [CO_2].

Estimation of growth and N uptake rate, and tissue N analysis

Mean whole-plant relative growth rate (R) between any two harvests was calculated as:

$$R = (\ln w_2 - \ln w_1)/(t_2 - t_1) \qquad (18)$$

where w_2 and w_1 are whole-plant dry weights at harvests 2 and 1, respectively, while $(t_2 - t_1)$ represents the number of days between the two harvests. Seedlings that were thinned at 11 DAP (approx. day 1 of the start of treatments) were used as initial biomass estimates for calculating RGR between days 1 and 19 (harvest 1). Plants used for photosynthesis measurement were harvested on day 19 and 39. Upon harvesting, roots were washed gently to remove the adhering sand, and the plants were divided into root, stem and leaf. These were oven-dried at 65°C to constant weights. The dried samples were weighed, and finely ground using a Foss Tecator Cyclotec Sample Mill (Model 1093, Sweden). The concentration of total-N in ground samples of root, stem and leaf tissue was determined using an elemental analyser (Fisons NA 2000, CE Instruments Inc., Milan, Italy).

Average N uptake rate, $\bar{v}$, between harvest intervals (also called specific N absorption rate) was calculated from whole plant tissue N gain and root RGR during the interval as:

$$\bar{v} = [(N_2 - N_1)/(R_2 - R_1)]R_{root} \qquad (19)$$

In eqn (19), N_2 and N_1 are the amounts of whole plant N at harvests 2 and 1, respectively, while R_2 and R_1 represent the corresponding root d.wt. R_{root} is the relative growth rate of the root calculated as in eqn (18).

All the data (except β) used for estimating R_{FB} using eqn (17) were derived from measurements. An estimate for β (the biosynthetic conversion efficiency, 0·6) was taken from the literature (see Gutschick, 1993). At the second harvest, α_L was not measured and values from harvest 1 were used.

RESULTS

Plant growth response to CO_2 and N

Whole-plant biomass increased approximately exponentially between days 1 and 39 at all levels of N and CO_2 (Fig. 1). By day 19, both CO_2-enrichment and increased N supply significantly increased whole plant biomass. At this stage, the effect of CO_2 on plant growth was independent of N supply. However, by day 39, the effect of elevated CO_2 on whole plant biomass depended on N supply (Fig. 1)—there was a significant CO_2 by N interaction.

During the first harvest (day 19), elevated CO_2 significantly increased RGR, by an average of 12 %, but there was no significant effect of CO_2 on RGR by the second harvest (day 39) (Table 1). Similar to CO_2, each additional N increment significantly enhanced RGR during the first

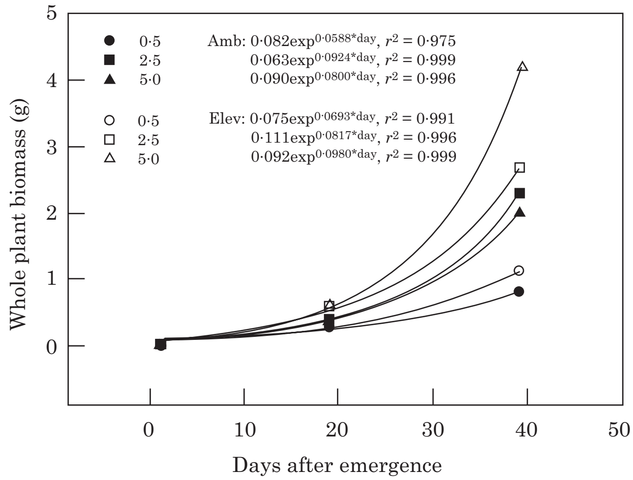

FIG. 1. Biomass accumulation in *H. annuus* 'Teddy Bear' grown at ambient or elevated CO_2, showing the strong dependence of long-term growth response to elevated CO_2 on N supply. Elevated CO_2 (open symbols), ambient CO_2 (closed symbols). 0·5 (●,○), 2·5 (■,□) and 5·0 mol m^{-3} N (▲,△).

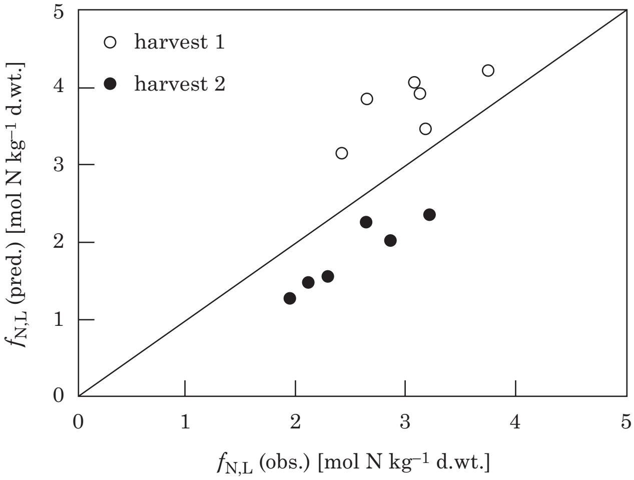

FIG. 3. Comparison of leaf nitrogen concentration predicted by the functional balance model [$f_{N,L}$(pred.)] with the measured leaf nitrogen concentration [$f_{N,L}$(obs)].

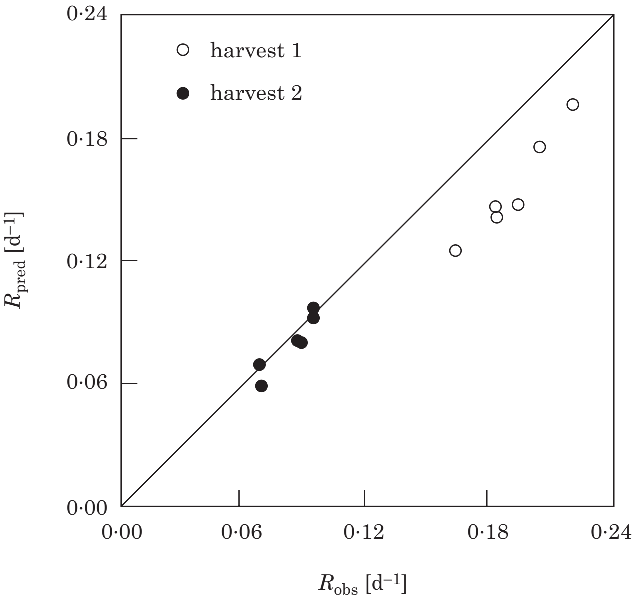

FIG. 2. Comparison of relative growth rate predicted (R_{pred}) by the functional balance model against measured relative growth rate (R_{obs}).

harvest, but unlike the CO_2 effect, the N effect persisted at the later harvest (Table 1).

Figure 2 shows the relationship between the observed RGR (R_{obs}) and RGR predicted (R_{pred}) by the model. At both harvests, there was excellent agreement between the observed and predicted RGR ($r^2 = 0{\cdot}94$, for harvest 1; $r^2 = 0{\cdot}90$, for harvest 2). By definition, functional balance is fully supported if there is a 1 : 1 relationship between predicted and observed RGR. At harvest 1, full functional balance was not supported, i.e. R_{obs} was significantly below the 1 : 1 line (Fig. 2). Nevertheless, the FB model accurately predicted the qualitative effects of both CO_2 and N supply on RGR. At the second harvest, however, FB between the root and shoot was attained as indicated by an almost 1 to 1 correspondence between R_{pred} and R_{obs} (Fig. 2). A further test of functional balance is the comparison of observed and predicted tissue nitrogen fractions. Here, we compared the observed leaf N fractions with that predicted by the FB model [eqn (13)]. Figure 3 shows that plants in the two harvests are on opposite sites of full functional balance. In particular, in harvest 2, $f_{N,L}$(obs) exceeds $f_{N,L}$(pred). This offset is expected if specific N uptake rates decline with plant age. The very good agreement of R_{obs} with R_{pred} in harvest 2 may be partly fortuitous, but mostly a confirmation of the FB model.

Responses of components of the FB model to CO_2 and N

Harvest 1. Photosynthetic N use efficiency, PNUE, or p^*, is a key component of the FB model that was used here to analyse RGR responses to CO_2 and N. This parameter was significantly affected by CO_2 but not by N supply (Table 1). In the FB model, p^* is expressed as the product of the CO_2-saturated PNUE, p_o, and the CO_2-saturation factor for photosynthesis, $C_{i\text{-}fac}$ [see eqns (16) and (17)]. Elevated CO_2 increased the $C_{i\text{-}fac}$ by 92 %, while overall p^* rose only 59 %. Examination of the components of p_o indicated that the offset was primarily due to a 24 % drop in $V_{c,max}$ at elevated compared to ambient CO_2. This analysis suggests that the effect of elevated CO_2 on p^* is mediated largely via the $C_{i\text{-}fac}$. Nitrogen supply had no significant effect on p^* despite a decline in p_o with increased N level (Table 1). Elevated CO_2 increased r (root to shoot ratio), but the effect was not significant ($P = 0{\cdot}22$). In contrast, increasing N supply significantly decreased r (Table 1). Specific N uptake rate, $\bar{v}$, increased with increased N supply and decreased with CO_2 enrichment, but the CO_2 effect was not statistically significant (Table 1). The N-partitioning ratio between leaf and whole shoot, α_N, decreased with increased N supply, but it did not rise significantly at elevated CO_2. Fractional allocation of shoot biomass to leaves, α_L, increased significantly with increased N supply but not with CO_2. However,

TABLE 1. *Morphological and physiological responses of* H. annuus *plants to CO_2 enrichment and N supply*

CO_2	N supply ($mol\ m^{-3}$)	$C_{i\text{-fac}}$	p_o	p^*	r	$\bar{v}$	α_N	α_L	R_{obs}
Harvest 1									
Amb.	0·5	0·211	33·59	7·094	0·487	0·014	1·435	0·806	0·163
Elev.	0·5	0·375	35·55	11·785	0·674	0·010	1·544	0·783	0·181
Amb.	2·5	0·199	35·01	7·091	0·419	0·021	1·291	0·828	0·182
Elev.	2·5	0·387	31·42	9·595	0·477	0·021	1·402	0·813	0·201
Amb.	5·0	0·189	24·78	6·258	0·325	0·039	1·164	0·820	0·193
Elev.	5·0	0·390	28·58	11·132	0·358	0·030	1·210	0·822	0·219
Significance									
CO_2-effect		*		*	ns	ns	ns	ns	*
N-effect		ns		ns	*	*	***	**	*
$CO_2 \times N$		ns		ns	ns	ns	ns	ns	ns
Harvest 2									
Amb.	0·5	0·179	37·37	6·679	0·321	0·004	1·219	—	0·069
Elev.	0·5	0·321	39·42	10·707	0·393	0·003	1·220	—	0·067
Amb.	2·5	0·169	34·38	6·671	0·298	0·008	1·316	—	0·094
Elev.	2·5	0·330	33·36	8·890	0·261	0·006	1·306	—	0·086
Amb.	5·0	0·165	26·93	5·668	0·183	0·015	1·133	—	0·088
Elev.	5·0	0·330	27·98	9·239	0·244	0·012	1·216	—	0·094
Significance									
CO_2-effect		*		*	ns	ns	ns		ns
N-effect		ns		ns	***	*	ns		*
$CO_2 \times N$		ns		ns	*	ns	ns		ns

$C_{i\text{-fac}}$, CO_2-saturation factor for carboxylation; p_o, CO_2-saturated photosynthetic N use efficiency (g photosynthate g^{-1} N d^{-1}); p^*, photosynthetic utility of N (g photosynthate g^{-1} N d^{-1}); r, root to shoot ratio; $\bar{v}$, specific N uptake rate (g N g^{-1} root d^{-1}); α_N, N-partitioning ratio to leaf; α_L, fraction of shoot biomass allocated to leaf. $^{***}P<0{\cdot}001$; $^{**}P<0{\cdot}01$; $^{*}P<0{\cdot}05$; ns, non-significant; —, not determined; $n = 3$.

the magnitude of α_L responses to N and CO_2 was very small (Table 1).

Harvest 2. The effects of CO_2 enrichment and N supply on $C_{i\text{-fac}}$ and p^* at harvest 2 were similar to those at harvest 1 (Table 1). However, at harvest 2, both $C_{i\text{-fac}}$ and p^* were consistently lower (17 % and approx. 11 %, respectively) than at harvest 1. As at harvest 1, elevated CO_2 increased $C_{i\text{-fac}}$ by 91 %, and p^* by 52 %. Again, the effect of elevated CO_2 on $C_{i\text{-fac}}$ was not fully translated into p^* due to a lower (32·5 %) $V_{c,max}$ in elevated than ambient CO_2 plants. As expected, allocation to the root significantly decreased with increased N supply as well as with age (*cf.* r at harvests 1 and 2, Table 1). The decrease in r from harvest 1 to harvest 2 appeared to be in part an allometric effect (Fig. 4). The apparent increase in r at elevated CO_2 was not significant ($P = 0{\cdot}09$). Similar to harvest 1, $\bar{v}$ responded markedly to N supply and to CO_2, but the 29 % decrease in $\bar{v}$ in response to elevated CO_2 was not statistically significant (Table 1). It also declined sharply with plant age (Table 1, *cf.* rates at harvests 1 and 2). The trends in measured V_{max} of NH_4^+ from harvest 1 to 2 (data not shown) were also qualitatively similar to $\bar{v}$, thus supporting the validity of using the latter as an integrated measure of uptake rate between harvest intervals. A decline in $\bar{v}$ with plant age appears to be a common plant response. According to eqn (2), a decline in $\bar{v}$ with aging may result either from a decline in R_{root} or an increased proportion of root biomass being allocated to secondary growth which is generally not active in ion uptake (see also Gutschick and Kay, 1995). At harvest 2, unlike at harvest 1, α_N, responded little to N or CO_2.

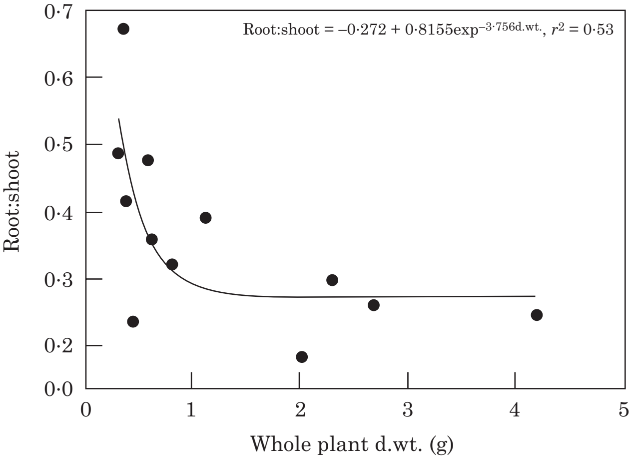

FIG. 4. Root-shoot biomass allocation in relation to plant size, showing the exponential decline of root : shoot ratio with an increase in plant size.

DISCUSSION

The results generally support the analyses of growth via functional balance, particularly in later growth, when the plants have apparently had adequate time to balance allocation with resource availability. This is evident in the agreement of RGR predictions with observations at harvest 2. Another way of evaluating the performance of the functional balance model is by comparing the observed and predicted [eqn (13)] leaf nitrogen fractions, $f_{N,L}$. The agreement of predictions with measurements for $f_{N,L}$ is, however, less satisfactory. Nonetheless, the model correctly predicted the qualitative pattern of changes in $f_{N,L}$ over time. In particular, the model predicted a higher and a lower $f_{N,L}$ at harvests 1 and 2, respectively, than were observed. The slight underestimation of $f_{N,L}$ at harvest 2 is consistent with the onset of limitation in N supply. Tissue N-fraction adjusts downward slowly, not instantaneously, as N uptake declines because the existing tissue comprises a large N reserve that upholds f_N. If this lagging adjustment to N limitation explains the discrepancies in f_N, it means that the excellent agreement of predicted *vs.* observed RGR is partly a result of some cancelling errors in our expression for RGR. There are factors in the R_{FB} expression that are moderately uncertain. Among these is leaf allocation, α_L, which was not measured at harvest 2.

The FB model reveals that photosynthetic N-use efficiency and N-uptake capacity have compensatory roles with regard to plant growth response to elevated CO_2. These compensatory roles are apparent in the data, and quantitatively expressed in the FB model, which resolves a factor $\sqrt{p^* \times \bar{v}}$ in RGR. To the extent that FB occurs (for example, harvest 2), it is seen that the rather substantial increase in p^* (over 50 %) itself translates to a modest increase in RGR (as a multiplicative factor of $\sqrt{1{\cdot}5}$ or about 1·22). Similarly, the drop in $\bar{v}$ translates to a modest lowering of RGR. Together, the two factors account for a modest change in RGR with elevated CO_2. This RGR change is, nonetheless, responsible for a large increase in biomass, which is proportional to $\exp^{RGR \times t}$, with t equal to time.

This study clearly showed that p^* increased in response to CO_2, but was unaffected by N supply. According to eqn (16), the changes in p^* result from the changes in the CO_2-saturation factor for carboxylation ($C_{i\text{-fac}}$) and in the CO_2-saturated PNUE (p_o). Analyses of these components indicated that the increased p^* response to elevated CO_2 resulted from increases in $C_{i\text{-fac}}$ since p_o was unaffected by CO_2. To our knowledge, the current study is the first to resolve the role of $C_{i\text{-fac}}$ as a mechanism leading to increased PNUE under elevated CO_2. The apparent lack of response in p_o may mask responses that could have occurred in its components. From our experimental data we computed p_o simply as a whole-leaf property, the light-saturated carboxylation rate (appropriate for high-light-grown plants) per unit N in the whole-leaf. We may, however, take p_o in turn as a pure p_{oo} for Rubisco enzyme, multiplied by the fractional allocation of N to Rubisco and fraction of Rubisco that is active in carboxylation:

$$\begin{aligned} p_o &= \frac{V_{c,\max(\text{leaf})}}{N(leaf)} \\ &= \frac{V_{c,\max(\text{active Rubisco})}}{N\ in\ Rubisco} \times \frac{N\ in\ Rubisco}{N\ in\ leaf} \\ &\quad \times \frac{Active\ Rubisco}{Total\ Rubisco} \end{aligned} \tag{20}$$

$$\equiv p_{oo} \times f(N\ in\ Rubisco) \times f(active\ Rubisco) \tag{21}$$

In view of eqns (20) and (21), the observed lack of response in p_o to CO_2 does not necessarily imply a lack of response in its components. Indeed, the latter two factors in eqns (20) and (21) can change in acclimation to CO_2 (e.g. Sage *et al.*, 1989). It is thus possible that leaf N allocation to Rubisco and the activation of Rubisco changed differentially with little or no net effect on p_o. Overall, however, the observed increased PNUE in response to elevated CO_2 is consistent with other reports (Drake *et al.*, 1997; Davey *et al.*, 1999). Peterson *et al.* (1999) also reported changes in PNUE under elevated CO_2. While they note that the local slope of assimilation (A) *vs.* N (dA/dN) is not changed significantly by elevated CO_2, a numerical analysis of their data reported in Tables A1 and Table A2 indicates that when PNUE is determined as A/N (rather than slope, dA/dN) the response to CO_2 is also positive. The lack of change in slope, dA/dN, may originate from repartitioning of N, but further research is required to determine this unequivocally.

The observed changes in $\bar{v}$ have some discernible origins and consequences. First, it is commonly recognized that elevated CO_2 accelerates plant growth (Coleman *et al.*, 1993). It is also known that plant nutrient uptake capacity declines with increasing plant age (e.g. Gutschick, 1993; Schenk, 1996; Gao *et al.*, 1998). Thus, it is likely that the decline in $\bar{v}$ at high CO_2 or with age (from harvest 1 to 2, Table 1) is partly a consequence of ontogenic drift. Second, $\bar{v}$ is determined not only by root kinetic parameters but also by soil diffusional limitation, which under the current experimental condition was probably the overriding factor. For example, the lowest-strength nutrient solution, 500 mmol m^{-3}, would saturate uptake kinetics as would stronger solutions, given that the Michaelis constants for NO_3^- or NH_4^+ uptake kinetics, K_m, are typically <150 mmol m^{-3} (see reviews in Glass and Siddiqi, 1995; Forde and Clarkson, 1999). Clearly, the changes in $\bar{v}$ indicate local depletion zones around fine roots must exist. Of course, addition of new solution temporarily reduces or eliminates these depletion zones, but they must re-form if bulk concentrations as high as 500 mmol m^{-3} can limit uptake and growth (Table 1). Consistent with the suggestion that, under the growth conditions of the current experiment, $\bar{v}$ was limited more by diffusion that root uptake capacity, BassiriRad *et al.* (1996) showed that NH_4^+ uptake rate from soil in loblolly and ponderosa pine was an order of magnitude lower than that determined from uptake solution. The increase in $\bar{v}$ with additional N (Table 1) is also consistent with the development of N-depletion zones around roots. Consequently, if $\bar{v}$ was diffusion-limited, any potential CO_2-effects would be minimized and this may largely explain the apparent unresponsiveness of $\bar{v}$ to CO_2.

Intuitively, biomass allocation to the root, *r*, should also be important in N acquisition and growth response to CO_2. However, we found little change in *r* in response to CO_2 (Table 1). This lack of response in *r* is apparently a more common occurrence than previously believed (Curtis and Wang, 1998; Norby *et al.*, 1999). The increase in RGR in response to elevated CO_2 was therefore accompanied by no changes in either *r* or $\bar{v}$. This was possible because of a compensatory increase in PNUE (Table 1). Such a compensatory mechanism may explain why elevated CO_2 generally enhances growth despite a substantial drop in leaf [N] (e.g. Curtis and Wang, 1998).

The FB model, in itself, does not predict the optimal values of r, $\bar{v}$, and other factors. Such predictions require other theoretical frameworks, such as optimality theory (Bloom *et al*., 1985; Hilbert, 1990; see also discussion of optimizing r and $\bar{v}$ in Gutschick and Kay, 1995) or mechanistic models from enzyme kinetics, gene expression, etc. (e.g. Stitt and Schulze, 1994). As Luo *et al*. (1999) note, these two frameworks rarely show predictive value in elevated-CO_2 studies, or have seldom been used to develop testable hypotheses. It is fair to state that (1) optimality theory assumes that selection pressures are well-known, and that physiological plasticity can be adequately elicited to enable plants to respond optimally to a complete range of environmental conditions (Gutschick, 1987), and (2) mechanistic theories require far more detailed and quantitative knowledge of kinetics and of gene expression than is currently available. Similar to optimality theory and mechanistic models, FB theory has seldom given rise to testable hypotheses nor has it been useful as a predictive tool (Luo *et al*., 1999). The FB model as formulated here overcomes some of the limitations of the previous FB models. Although we do not propose that FB theory predicts all the responses in r, $\bar{v}$, f_N, etc. it can be used to link the various components of the model. Consequently, it serves as an important tool to evaluate the relative effects of these components on plant growth response to elevated CO_2. From such analyses, we find that adjustments in p^* and $\bar{v}$ have considerably more effect on RGR than do adjustments in r. An additional advantage of the FB model, as presented here, is the ability to formulate new hypotheses. For example, it can be hypothesized that adjustments in r have little effect on growth responses to elevated CO_2.

We reiterate that increased r appears relatively unimportant, particularly because adjustments in PNUE are both larger and more potent in influencing RGR. To amplify the point, the FB model predicts that r enters in RGR as the factor $\sqrt{r}/(1+r)$. Thus, for example, the largest relative change in r, 38 % (from 0·487 to 0·674, see Table 1) should increase RGR by only 4 %. Using a simulation model study of prairie grasses, Hunt *et al*. (1998) also found that growth response to CO_2 was insensitive to changes in root allocation. Similarly, Hilbert *et al*. (1991) using a cost-benefit model analysis predicted that the optimum r that maximized whole plant RGR was insensitive to increases in [CO_2].

CONCLUSIONS

An accurate prediction of plant responses to elevated levels of CO_2 requires a better understanding of uptake and allocation of resources that influence growth. In this context, plant demand for N and the capacity to acquire it, and how these are influenced by elevated CO_2 all need to be considered collectively. In this analysis, we evaluated the utility of the modified FB model of Gutschick (1993) for predicting whole plant growth responses to elevated CO_2. The model relates RGR to key morphological and physiological plant properties such as root to shoot ratio, specific root N uptake rate, PNUE, and allocation of biomass and N between leaf and shoot. The more direct effect of CO_2 is incorporated in the model as a component of PNUE via the C_i-factor, which is the CO_2-saturation factor for carboxylation. The predicted whole plant RGR responses to elevated CO_2 showed excellent agreement with observations. This was particularly the case in later growth stages when there was sufficient time for the early transient root and shoot responses to adjust to the prevailing resource levels in the respective environments. The success of the model in predicting growth response to CO_2 can be exploited for identifying (1) mechanisms and (2) the relative effects of the different plant attributes in determining growth responses. With respect to (1), the model showed that RGR responded to CO_2 despite a decreased specific N uptake rate, because of a substantial rise in PNUE that more than compensated for the drop in $\bar{v}$. Regarding (2), the model analysis revealed that plasticity in r was relatively unimportant in determining RGR response to elevated CO_2, but it had a substantial influence on tissue [N]. Intuitively, the effect of r on tissue [N] should translate to a growth effect since growth response to CO_2 is considered to depend on N acquisition (Bazzaz, 1990; Poorter, 1998; Stitt and Krapp, 1999). There is evidence that specific N uptake rate is inversely related to root size (e.g. Lainé *et al*., 1993). Thus, the FB model formulated here, by incorporating morphological and physiological properties, provides a sound framework for understanding whole plant growth response to rising levels of atmospheric CO_2.

ACKNOWLEDGEMENTS

We thank the greenhouse staff of the University of Illinois at Chicago for help during the experiment. Two anonymous referees provided comments that improved the manuscript. This work was supported by the United States Department of Energy contract #DE-FG02-97ER62332 and the National Science Foundation Division of Environmental Biology grant number IBN-9728785 to H.B.

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APPENDIX

Symbol	Meaning [unit]
$\bar{A}$	Mean assimilation rate per leaf area [g glucose m^{-2} d^{-1}]
α_L	Fraction of shoot dry mass that is in leaves [g g^{-1} or unitless]
α_N	N-partitioning ratio to leaf [g g^{-1} or unitless]
a_{pl}	Plant leaf area [m^2]
β	Biosynthetic conversion efficacy [g dry matter g^{-1} glucose]
C_i	Leaf intracellular CO_2 partial pressure [Pa]
$C_{i\text{-fac}}$	C_i-factor, the CO_2-saturation factor for carboxylation [Pa Pa^{-1} or unitless]
$f_{N,L}$	Leaf N fraction [%, g N g^{-1} leaf DM]
$f_{N,W}$	Fractional N content in whole plant [g N g^{-1} dry matter or unitless]
Γ	CO_2 compensation partial pressure [Pa]
K_{co}	Effective Michalis–Menten constant for CO_2 [Pa]
$m_{L,a}$	Mass per leaf area [g dry matter m^{-2}]
m_{pl}	Whole-plant mass [g]
m_r, m_s	Mass of root or shoot [g]
$\dot{M}_{CL}$	Carbon-limited growth rate [g dry matter d^{-1}]
$\dot{M}_{NL}$	Nitrogen-limited growth rate [g dry matter d^{-1}]
$\dot{M}_N$	Rate of gain in mass of N [g N d^{-1}]
N_a	Nitrogen content per leaf area [g N m^{-2}]
p^*	Photosynthetic utility of N [g photosynthate g^{-1} N d^{-1}]
p_o	CO_2-saturated photosynthetic N use efficiency [g photosynthate g^{-1} N d^{-1}]
r	Root to shoot ratio [g g^{-1} or unitless]
R, R_{FB}	Relative growth rate [g g^{-1} d^{-1}], RGR at functional balance between shoot and root
R_{CL}, R_{NL}	Carbon-limited and nitrogen-limited RGR
$\bar{v}$	Average specific N uptake rate [g N g^{-1} root d.wt. d^{-1}]
$V_{c,max}$	Rubisco carboxylation capacity [μmol m^{-2} s^{-1}]