

## Extended model of functional balance

### Utility of functional balance models: the original model

Plants are subjected to widely-varying nutrient concentrations, diverse patterns of spatial distribution of nutrients in soil, and varied schedules of nutrient availability. Presumably to maximize growth and, ultimately, reproductive success = Darwinian fitness, they adapt to such diverse conditions with changes in genetics (ecotypic differentiation, speciation) and in phenotype (physiology, morphology, phenology). Here, as in two articles on our original functional balance model (Gutschick, 1993; Gutschick and Kay, 1995), we focus on physiology and morphology: how do plants acclimate in root uptake capacity for nutrients ( $V_{\max}$ ,  $K_m$  in the Michaelis-Menten formulation of uptake rate per area or mass of roots,  $v = V_{\max} c/(c+K_m)$ ), allocation to roots (as root:shoot ratio,  $r$ ), and tissue nutrient content? The discussion can also be extended to root morphology, *e.g.*, to root fineness that increases surface:volume ratios (see refs. above.). One may ask, further, how much each response contributes individually to maximizing relative growth rate (taken as a potent index of fitness).

In the two papers cited, we took the approach of formulating relative growth rate (RGR) in terms of  $r$ ,  $v$  (or components  $V_{\max}$ ,  $K_m$ , and nutrient conc. at the root surface,  $c_a$ ), and descriptors of how nutrients are used for photosynthesis and growth: the photosynthetic nutrient-use efficiency,  $p^*$  (in units such as grams of photosynthate fixed per gram of N per photoperiod or day), the fractional allocation of shoot mass to leaves ( $\alpha_L$ ), and the efficiency of biosynthesis (fractional conversion of photosynthate to final dry matter,  $\beta$ ). A full derivation is given in both papers. Briefly, we formulate RGR in two ways: a rate limited by nutrient availability,  $RGR^{nl}$ , and a rate limited by the use of that nutrient (commonly, N) in photosynthesis,  $RGR^{Cl}$ . In steady exponential growth (early growth), these two rates must equal each other. This, in fact, sets the tissue nutrient concentration,  $f_n$ . This concentration is thus dependent on the balance between gain and use of nutrient, and therefore on  $r$ ,  $v$ ,  $p^*$ ,  $\alpha_L$ , and  $\beta$ . Before discussing other implications, we complete a quick derivation of the original functional balance (FB) model: a

$$\begin{aligned}
 RGR^{nl} &= \frac{\text{(rate of dry matter increase)}}{\text{(current dry mass)}} & (1) \\
 &= \frac{\text{(rate of nutrient gain by whole plant)}}{\text{(fractional content of nutrient in dry matter) \cdot (plant dry mass)}} \\
 &= \frac{m_r v}{f_n(m_r + m_s)}
 \end{aligned}$$

Here,  $m_r$  and  $m_s$  are the root and shoot dry masses, respectively. We can simplify this by dividing numerator and denominator by  $m_s$  and using  $r = m_r/m_s$ :

$$RGR^{nl} = \frac{r v}{f_n(1 + r)} \quad (2)$$

The other rate is

$$\begin{aligned}
\text{RGR}^{\text{Cl}} &= \frac{\beta \text{ (rate of photosynthesis by whole plant)}}{\text{(current dry mass)}} & (3) \\
&= \frac{\beta \text{ (total leaf dry mass)}(\text{PS rate per dry mass})}{m_r + m_s} \\
&= \frac{\beta \alpha_L m_s p^* f_n}{m_r + m_s} \\
&= \frac{\beta \alpha_L r p^* f_n}{1 + r}
\end{aligned}$$

Both rates involve  $f_n$ ; equating the two rates gives us an expression for  $f_n$ ,

$$f_n = \sqrt{\frac{r v}{\beta \alpha_L p^*}} \quad (4)$$

We can then eliminate  $f_n$ , substituting it into either RGR formula, to get

$$\text{RGR}^{\text{FB}} = \frac{\sqrt{r}}{1+r} \sqrt{v \beta \alpha_L p^*} \quad (5)$$

The formula of Eq. (5) has both strengths and constraints. It shows how traits or parameters such as  $r$  and  $v$  can affect RGR. The "leverage" of  $r$ , in particular, is shown to be small, since the factor  $\sqrt{r}/(1+r)$  varies rather slowly as  $r$  changes radically. The leverage of uptake rate,  $v$ , is as the square root, which is rationalized in the papers. A consequence is that RGR increases only 5% when  $v$  increases by 10%. The effect of high  $\text{CO}_2$  can be estimated, by noting that this increases  $p^*$ ; RGR is predicted to increase and  $f_n$  to decrease. The exact quantitative changes in RGR and  $f_n$  depend upon how  $r$ ,  $v$ ,  $\alpha_L$ ,  $\beta$ , and  $p^*$  behave. The FB model cannot predict such changes. Likewise, other, more mechanistic models are not ready for such predictions, either, particularly from deep bases such as gene expression, which requires vast amounts of information not available for any plant yet. Optimization models may be useful: one seeks the mathematical values of  $r$ ,  $v$ , etc. that maximize RGR. Such attempts are discussed in both cited papers. Some results are remarkable, including that  $r$  should be 1.0, independent of nutrient status, and that  $v$  should be as large as physically possible (limited only by space for uptake carrier proteins on root surfaces, for example). These predictions are rarely borne out. Optimization models have implicit assumptions that plant plasticity is unlimited and that RGR is the proximate goal for maximization. Failures of optimization models teach us to search for other aspects of growth that might be the main subject of natural selection pressures, among other things. In any event, the FB model provides a first estimate for how traits such as  $r$ ,  $v$ , and  $p^*$  contribute individually to RGR. It also indicates how responses might compensate each other, such as increases in one with decreases in another, and also how responses such as increased  $p^*$  in high  $\text{CO}_2$  lead naturally to low  $f_n$  but not inherently penalizing RGR.

One limitation of FB models in general is that the growth stage being analyzed must be balanced or nearly so. Big changes in nutrient availability cause progressive changes in uptake and use of nutrients, such that these two processes may be imbalanced (modestly) for long times. For example, a progressive decrease in nutrient availability will cause nutrient

uptake to lag the photosynthetic use of the nutrient. The equating of two RGR's is not valid, though it may be close to valid if the change is not abrupt.

### The need for an extended model

A second limitation of the original FB model is that the role of mycorrhizae (MR, or MRF to denote the mycorrhizal fungus specifically) is not resolved, while MR may be quite important, particularly in responsiveness of growth to high CO<sub>2</sub>. The MRF can change (usually increase)  $v$ , while also using photosynthate. The uptake rate per unit mass or MRF, denoted as  $v_M$ , may be larger than that of pure root tissue. The metabolic rate per unit mass may also be larger, particularly if we account for root exudation as a usage of photosynthate. The effects of MR on  $v$ ,  $\beta$ , etc. can be formulated several ways, each with various limitations or approximations. Consider first the carbon-limited growth rate. One might equate the rate of photosynthate supply (assimilation rate,  $A$ ) with the sum of rates of use for (1) carbon skeletons in dry matter, (2) energy metabolism to generate ATP for polymer synthesis and nitrate reduction, etc., and (3) consumption by MRF, as an "operational" (non-growth) cost proportional to existing MRF biomass,  $m_M$ . Taking the dry mass of C-skeletons as closely equal to that in original photosynthate, and taking both of the first two PSate uses as proportional to the rate of new tissue construction, we have:

$$A = (1 + R_g) \frac{dm_p}{dt} + R_M m_M \quad (6)$$

We may take MRF biomass as proportional to plant biomass, to express the entire right-hand side in terms of plant biomass. However, because plant biomass  $m_p = m_s + m_r$  and its time derivative are mixed together, the resulting equations for growth rate and RGR become rather involved algebraically. The basic formulation is also somewhat unrealistic, given that MR fungi also grow. A better formulation is probably to treat plant tissues and MRF tissues similarly. We ascribe to each a consumption of photosynthate proportional to their biomass growth rates. Other formulations of growth (see, e.g., modelling compendia such as Johnson and Thornley, 1988) resolve a maintenance component of respiration that consumes photosynthate at a rate proportional to biomass, similarly to the third term in Eq. (6). This would lead to the same complications in algebra. For a cleaner treatment, we consider that maintenance costs typically rise in parallel with growth rate; more active tissues have higher maintenance rates. Thus, we approximate maintenance PSate usage as  $(R_m = \text{constant}) * dm_p/dt$ . We can then write

$$A = (1 + R_g + R_m) \frac{dm_p}{dt} + (1 + R_{g,MR} + R_{m,MR}) \frac{dm_M}{dt}. \quad (7)$$

For a given tissue composition (fraction of carbohydrate, protein, and lipid), the coefficients are nearly constant with time. We can combine them and write

$$A = \frac{1}{\beta_p} \frac{dm_p}{dt} + \frac{1}{\beta_M} \frac{dm_M}{dt}. \quad (8)$$

Here,  $\beta_p$  is the biosynthetic conversion efficiency for plant tissue growth, just as in the original model;  $\beta_M$  is the equivalent for MRF tissue. Let us take the inverse of this last quantity, which is the photosynthate demand for growth of MRF tissue, as a factor  $G$  times the that for

plant tissue. We expect that  $G > 1$ , reflecting some wasteful metabolism, as of exudates. The value of  $G$  may change with phenological stage but should be reasonably constant in one or more growth stages of interest. This proposal may be taken as a hypothesis that is useful to test, particularly for its implications in growth models such as we are developing here.

Let us express the biomass allocation to MRF as a multiplier  $\alpha_M$  applied to root-only biomass,  $m_M = \alpha_M m_r$ , with  $\alpha_M$  commonly much less than 1. We then write the carbon-limited growth rate as

$$\frac{dm_p}{dt} = \frac{\beta A}{(1 + G\alpha_M \frac{r}{1+r})}. \quad (9)$$

Here, we have dropped the subscript "p" on  $\beta$ , which is understood as applying to plant tissue. This leads to a formulation of relative growth rate as

$$RGR^{Cl} = \frac{\beta \alpha_{LP}^* f_n}{(1+r)(1 + G\alpha_M \frac{r}{1+r})} = \frac{\beta \alpha_{LP}^* f_n}{(1+r)[1+G\alpha_M]} \quad (10)$$

Similarly, we need to express the effect of the presence of MRF fungi on whole-plant uptake. We write this uptake rate in terms of rates per unit dry mass of plant root and of MRF, as  $m_r v_p + m_M v_M$ . Expressing the mass of MRF as  $\alpha_M m_r$ , we ultimately arrive at an expression for nutrient-limited relative growth rate,

$$RGR^{nl} = \frac{r(v_p + \alpha_M v_M)}{f_n(1 + r(1 + G\alpha_M))}. \quad (11)$$

Just as for the original FB model, we can solve for  $f_n$  and then for  $RGR^{FB}$ .

Some interesting predictions arise. If we write  $v_M$  as a multiplier times the root specific uptake rate,  $v_M = g v_p$ , we obtain

$$RGR^{FB} = \sqrt{\frac{\beta \alpha_{LP}^* r v_p (1 + g\alpha_M)}{[1 + r(1 + G\alpha_M)][1 + r(1 + \alpha_M)]}} \quad (13)$$

The ratio of this RGR to that without MRF present is

$$\frac{RGR_{MR}^{FB}}{RGR^{FB}} = (1+r) \sqrt{\frac{(1 + g\alpha_M)}{[1 + r(1 + G\alpha_M)][1 + r(1 + \alpha_M)]}} \quad (14)$$

In the case that  $r = 0.5$ ,  $g = G = 3$ , and  $\alpha_M = 0.3$ , this ratio is 1.15. That is, adding MRF that are "more active versions" of roots, in uptake but also in using up photosynthate, the RGR is improved by a factor of 1.15, or a 15% relative gain. The expression for  $f_n$  shows a gain in  $f_n$  from the presence of MRF, a gain greater than that from the acceleration of uptake only, because the MRF's consumption of carbohydrates reduces the C-skeleton base of total dry mass.

### **Second consideration: carbon use, inhibition of photosynthesis, and response of RGR to carbohydrate status**

It has been suggested (ref.) that the use of photosynthate by MRF might relieve some

inhibition of photosynthesis from product accumulation (ref. - Foyer?), when plants grow at high CO<sub>2</sub>. While it seems unlikely that any "waste" of photosynthate would improve RGR (barring some unusual forms of photosynthesis response to carbohydrate status), the consequences of photosynthate use and of photosynthesis inhibition do merit attention.

For this task we must formulate a carbohydrate balance. Let A = photosynthetic rate of the whole plant be modified by a factor 1/(1 + k<sub>C</sub>f<sub>C</sub>), where f<sub>C</sub> is a measure of (average) whole-plant sugar content, such as molarity of sucrose in plant tissue. Here, k<sub>C</sub> is an (empirical) inhibition constant. We write

$$A = \alpha_L m_s p^* f_n \frac{1}{1 + k_C f_C}. \quad (15)$$

This is a modified source term for carbohydrates. To determine f<sub>C</sub>, one must have a description of how photosynthate use for growth responds to f<sub>C</sub>. The relative growth rate does respond to carbohydrate availability (ref.). If we take a simple Michaelis-Menten form,

$$RGR = RGR_{\max} \frac{f_C}{f_C + K_e}, \quad (16)$$

we can equate this to the C-limited RGR, to obtain

$$\frac{\beta \alpha_L p^* f_n}{[1 + r(1 + G\alpha_M)][1 + k_C]} = RGR_{\max} \frac{f_C}{f_C + K_e}. \quad (17)$$

For known values of β, α<sub>L</sub>, etc., we can solve for f<sub>C</sub>. That is, we have a second functional balance, now involving carbohydrate gain and carbohydrate use. We must solve this functional balance (FB) equation simultaneously with the FB equation for nutrient uptake and use. It is possible to obtain analytical expressions (closed mathematical forms, algebraic expression and square roots). However, these are very complicated and difficult to comprehend. For our purposes, we use a conceptually simple approach. We estimate f<sub>n</sub> in Eq. (17) and solve for f<sub>C</sub>, thus, for RGR<sup>Cl</sup>. In general, this will not equal RGR<sup>nl</sup>, so we progressively alter f<sub>n</sub> until F = RGR<sup>Cl</sup> - RGR<sup>nl</sup> equals 0 as closely as we wish to approximate it. Simply, we begin with extreme limits of the range in which f<sub>n</sub> might lie (say, 0.01 = 1% N to 0.06 = 6% N). At each limit, we compute the RGR difference, F. It will be positive at too-high f<sub>n</sub> and negative at too-low f<sub>n</sub>. We evaluate F at the midpoint and then determine if the solution F=0 lies in the upper half of the interval or the lower half (the sign of F must change between the new upper and lower limits of f<sub>n</sub>). We keep subdividing the interval in which f<sub>n</sub> is found to lie, to any desired accuracy. Only a dozen or so halvings will typically reduce the uncertainty in f<sub>n</sub> to 0.00001.

The formulation here introduces a maximal RGR, for which there is evidence (Poorter?). This quantity is not typically measured, nor easily measured, but its magnitude has some significant effects on RGR responses to MRF presence, to high CO<sub>2</sub>, and to altered nutrient availability. The significance can be explored readily by running the computer version of the model. So, too, the computer model can be used to explore many other potential couplings of plant responses and their leverage over RGR and over nutrient content f<sub>n</sub>.

## Running the model

The model is coded in Fortran 77 and has been compiled on a Sun IPX. The source code can be downloaded from my ftp site, bilbo.nmsu.edu, using an anonymous ftp login:

```
ftp bilbo.nmsu.edu
(System types:) Connected to bilbo.nmsu.edu
                220 bilbo FTP server (Sun OS 4.1) ready.
                Name (bilbo.nmsu.edu:vince):
(You type:) anonymous
(System types:) 331 Guest login ok, send ident as password.
(You type:) [your email address]
                (Don't worry, I won't sell your listing to Time/Warner/AOL)
(System types:) 230 Guest login ok, access restrictions apply.
ftp>
(You type:) cd pub
(You type:) get FB2.f
(You type:) quit
```

You can also run the program directly on my computer, using a guest account. Use telnet to gain access:

```
telnet bilbo.nmsu.edu
(System types:) Trying 128.123.5.8 ...
                Connected to bilbo.nmsu.edu.
                Escape character is '^]'.

                Sun OS UNIX (bilbo)

                login:
(You type:) guest
(System types:) Password:
(You type:)xxxxxxx [the password will be available by email
                    send to vince@nmsu.edu]
(System types out a 'message of the day,' which you can ignore,
and then types:) bilbo{guest}nn:
                (Here, nn is the number of the command; if you're familiar with
                the use of history in the UNIX C-shell, this can be useful)
(You type:) FB2.ex
```

At this point, the Fortran program takes over, prompting you for all the information needed by the model:

```
alphaL =  $\alpha_L$ 
pstar = p*
r
beta =  $\beta$ 
vplant = v
alphaM =  $\alpha_M$ 
vM = vM
kC = kC
Ke = Ke
```

G

$RGR_{max} = RGR_{max}$

$fN_{lo0}$ ,  $fN_{hi0}$  = lower, upper limits of  $f_n$  in which to search for solution

The program prints out this list, with the definition of each term and the units to be used. It then prompts you to enter each parameter value. For some parameters, there are options. For example,  $p^*$  can be entered directly in the units used by the model, which are grams of photosynthate per g of N in leaves per day (that is, per photoperiod). It can also be computed from average daytime assimilation rate per unit mass ( $\mu\text{mol}_{\text{CO}_2}\text{g}^{-1}\text{s}^{-1}$ ),  $f_n$  of leaves ( $\text{g}_{\text{N}}\text{g}_{\text{DM}}^{-1}$ ), and the photoperiod length, in seconds. The prompts allows you all the options for specifying  $p^*$ ,  $v$ , and  $v_M$ .

After you have entered values for all parameters, you have several options:

run = run a simulation to compute RGR and  $f_n$

see = see the numerical values you have chosen for all parameters

all = re-enter all parameter values

stop = stop execution and exit the program

*name* = change the value of parameter you have named. For example, if you type *vplant*, the program will prompt you to enter a (new) value for  $v$ . This option is handy for making multiple runs, changing parameters and comparing RGR (and  $f_n$ ) between runs. It is also useful to correct mistakes you have made, before attempting a run that (rarely) may cause the program to abort.

I have attempted to make the system robust, so that no erroneous entries will cause the program to stop; rather, the program recognizes an error, notifies you, and asks you to re-enter the value or the choice of option. In the rare cases that the program aborts, you may re-start it by typing *FB2.ex* again when the prompt *bilbo{guest}nn:* appears again. In the even rarer case that the program hangs up (answers are normally virtually instantaneous), you can type a ^C (control-C) to kill the program and start over.

The list of plant parameters demanded by the model is moderately extensive and includes some parameters not commonly measured, such as  $RGR_{max}$ , or metabolic demand of MR fungi. The user is encouraged to make informed estimates of parameters not measured. One might use somewhat noisy experimental data, such as data on growth that might indicate biosynthetic conversion efficiency and, thus, G. One might also use parameters from other studies on the same species or functional group. Finally, one might make educated guesses, such as that  $RGR_{max}$  has not been found to exceed 0.2 per day in the functional group of plants under study. The estimates of these parameters do have functional significance in the model. We take this as an encouragement to learn more about genetic and environmental controls on these parameters, and to design future experiments with the model demands in mind. The parameters are of use in many other models or analyses, as well.

### Other features of the program

In addition to reporting the computed RGR and  $f_n$ , the program reports the plant carbohydrate status ( $f_C$ ) and the consequent degree of inhibition of photosynthesis.

If one changes a single parameter, the program will also compute the change in RGR and the sensitivity of RGR to that parameter. Sensitivity is defined as the logarithmic derivative,

d (ln RGR)/d(ln parameter), and it has no dimensions (is a pure number, that is). Basically, it represents the ratio of the relative change in RGR to the relative change in the parameter. For example, in the original model, RGR varies as the square root of uptake rate  $v$ . The sensitivity is then 1/2; a 10% change in  $v$  should give a 5% change in RGR (say, from 0.2 to  $1.05 \cdot 0.2 = 0.21$ ). For the extended model, the sensitivity of RGR to the various plant parameters is not readily calculated from analytic formulae. The program computes the derivative numerically, as

$$\text{Sensitivity} = \frac{\text{value}_{\text{old}}}{\text{RGR}_{\text{old}}} \frac{(\text{RGR}_{\text{new}} - \text{RGR}_{\text{old}})}{(\text{value}_{\text{new}} - \text{value}_{\text{old}})} \quad (18)$$

The sensitivity of RGR to some parameters is rather low, and this may be counterintuitive. For example, RGR is insensitive to root:shoot ratio,  $r$ , in the original model and in this extended model. One can calculate the sensitivity  $S(r)$  as  $0.5 \cdot (1-r)/(1+r)$  in the original model (calculus not shown). For  $r = 0.5$ , this gives  $S(r) = 0.17$ , far less than 1.

The program also reports calculations of sensitivity of  $f_n$  to the parameter being varied. In one example I ran recently, the sensitivity (call it  $S$ ) of  $f_n$  to  $p^*$  was -0.397. That is, an increase of 10% in  $p^*$  causes a reduction of 4% in  $f_n$  (from  $0.047 = 4.7\%$  to

$$(1 + S \cdot [\text{relative change in } p^*]) \cdot (\text{old } f_n) = (1 + [-0.397][0.1]) \cdot 0.0470 = 0.0451 \quad (19)$$

Often, the model (and experimental reality) indicate that RGR is rather insensitive to a parameter, while  $f_n$  is not. For example, RGR is often very insensitive to  $r$ , while  $f_n$  is very sensitive. In an example I ran recently,  $S(r)$  was 0.10 for RGR but 0.58 for  $f_n$ .

The program can also calculate the sensitivity of root uptake rate  $v$  to root uptake parameters  $V_{\text{max}}$  and  $K_m$ , as well as to nutrient concentration in bulk soil (away from local depletion zones around roots). This requires that one has chosen to compute  $v$  from these parameters and from the root radius and inter-root spacing. The program computes  $v$  using the steady-state concentration gradients from bulk soil to root, assuming that roots are long uniform cylinders (at least over their zone of active uptake). Some sensitivities of  $v$  are also remarkably small. When diffusivity of the nutrient in soil is low, increased  $v$  or decreased  $K_m$  by roots may have very little effect;  $S$  can be 0.05, for example, far from 1 as expected in models that do not resolve the limitation of uptake by diffusion in soil.

## Final remarks

I welcome comments about the use of the model and of conceptual development. If alternative formulations of how physiology operates should be considered, please communicate with me. My contact information is:

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