

## Nutrients and limiting factors

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### Abstract

The terms 'nutrient' and 'limiting factor' summarise the results of an experiment in which increase in supply results in an increased response. By extension they are often – perhaps usually – used when the user believes that were such an experiment made it would have this characteristic. If the supply is further increased the response diminishes and may, eventually, become negative. 'Nutrient' and 'limiting factor' therefore apply, strictly, only when the circumstances are specified: they cannot be attached to a particular substance without qualification. The claim that 'nitrogen is a nutrient (or limiting factor)' is incomplete. All nutrients are limiting factors, but the reverse is not true. The widespread belief that only one factor can limit a complex process at one time is demonstrably false in general, though it may sometimes be true in particular cases.

### Introduction

Suppose that an experiment is made to reveal the way in which an organism responds to the supply of a substance. Examples are shown in Fig. 1 from Austin & Austin (1980). Grasses of several species were grown in plots in monoculture or in five-species mixtures with equal numbers of each species. They were watered at fixed intervals with a solution of defined composition. The proportions of solutes were always the same but the overall concentration differed from 1/64 to 16 times the arbitrary standard: a range of 1024-fold. In this case the 'supply' is shown as the relative concentration but should be understood to indicate the total supplied to the grass roots over the course of the experiment. The 'response' is given as the shoot dry mass per pot, multiplied by 5 in the case of the mixtures in order to make monoculture and mixture results

comparable. The response is thus a yield relative to an arbitrary standard. Each graph shows a large part of the 'response curve'. It also shows the difference between the response when a species is competing with itself only and the 'ecological response' when it is competing with others. The original article called the first response curve, with self-competition, a 'physiological response'. One might expect there to be a single physiological response curve in a given physical and chemical environment, but innumerable ecological response curves for arbitrary mixtures of other species. Fig. 1 shows that for the robust species, *Dactylis glomerata*, there is little difference between the physiological and the ecological response curves, but for the much smaller species, *Vulpia membranacea*, the two curves are very different both in size and in the location of the peak.

In what circumstances are the solutes 'nutrients'? The answer is very simple and is the touchstone for all such experiments: *if an increase in supply results in an increase in response then the solute is, in the conditions of the experiment, a nutrient*. This could, of course, be cast in terms of a diminished response as the supply is reduced. The essential feature is that, in graphic terms, both correspond to the condition that the line connecting two points on the response curve

The Symposium 'Nutrient Cycles: a Joy Forever' was held in Delft on 19 May 1993 in honour of Han Golterman on his official retirement. I there described the processes of carbon cycling in peat accumulating systems and compared them with those operating in lakes. Accounts of these processes are already available, however (Clymo, 1984, 1987, 1991, 1992a, 1992b), so I therefore consider here a quite different but equally relevant topic which should just as effectively recall to Han many cheerfully fierce discussions during our thirty-year friendship.

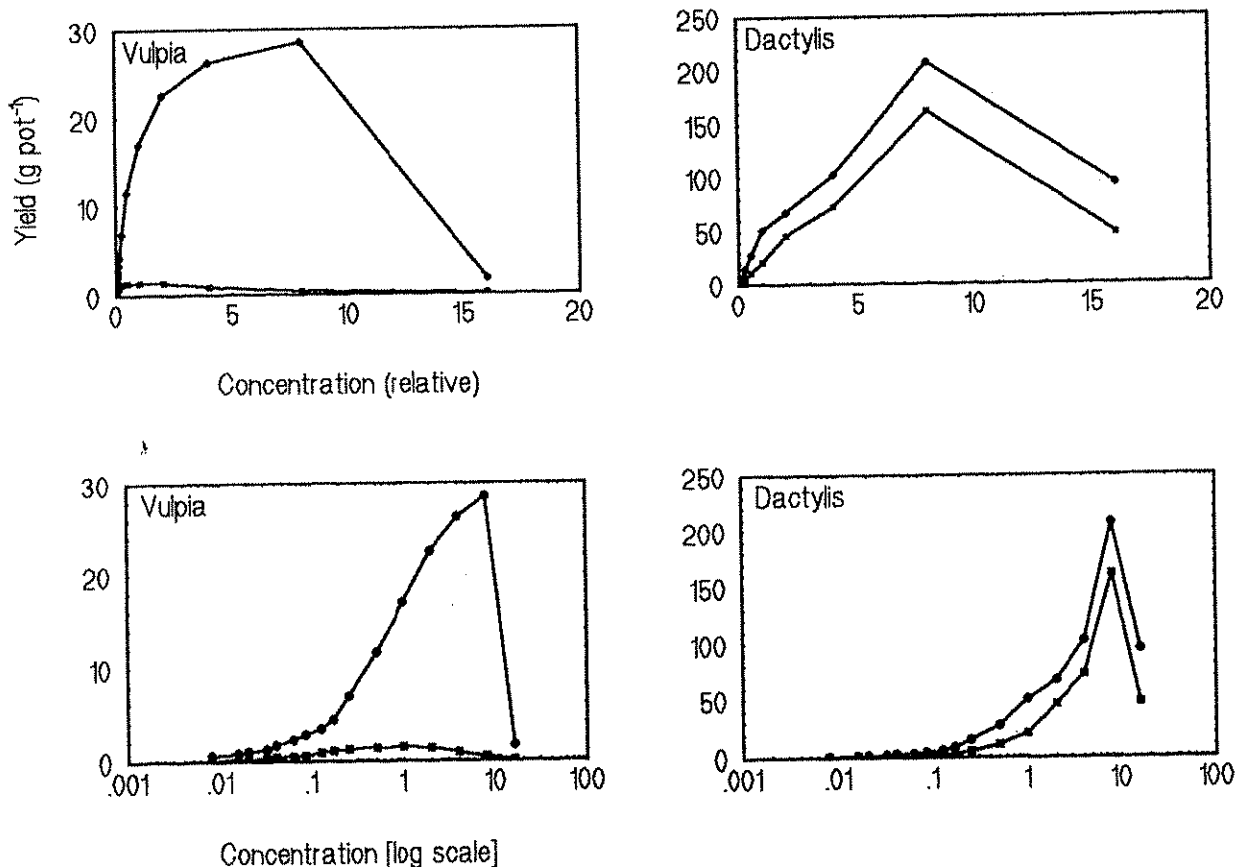


Fig. 1. Yield of two grass species (*Vulpia membranacea* at left; *Dactylis glomerata* at right) in response to supply of a mixture of solutes. The proportions of solutes were fixed. The scale values show the total amount supplied relative to an arbitrary standard. The right side yield scale is about 8-fold coarser than the left side. The lower graphs show the same data as the upper ones but on a logarithmic supply axis. Each graph shows an upper curve for the yield when the grass was grown in a pure stand; the lower curve is for the nominal species as 20% of the plants in a mixture with four other species each being 20% of the whole mixture. Results from Austin & Austin (1980).

has a positive slope. What then of the upper parts of the supply range in which the lines have negative slopes? In these regions the solute is not behaving as a nutrient: it has become a poison or toxin. The property of being a nutrient is rather obviously not attached to the solute unconditionally, or even to the same range of supply for all species (as Fig. 1 shows). Thus the ex-cathedra statement that 'nitrate is a nutrient' is sloppy at best and in many circumstances no more than a guess that, were the experiment to be made, results similar to those in Fig. 1 would be found. Even looser is the description of phosphate (for example) as a 'mineral nutrient', for a mineral is a solid with at least a generic chemical composition and a specific crystal structure while what is usually intended is phosphate in solution.

There is quite a number of published examples showing the range of the supply in which a solute behaves as a nutrient, often followed by a zone in which

the response is flat. There is no commonly accepted term for this range of supply where the response is flat, but 'neutral' or 'saturation' is sometimes used. There are surprisingly few published examples that show all or most of the response curve, as Fig. 1 does. They are probably much fewer than the textbook generalisations. The top half of Fig. 1 shows that the neutral range in the conditions of the experiment is narrow relative to the nutrient and toxic ranges. In the lower half of Fig. 1 the same results are plotted but with supply on a logarithmic scale: the difference in appearance is striking, but the conclusions are unchanged.

#### Limiting factors

The concept of limiting factors developed during the nineteenth century in practical applications to agri-

culture. Mitscherlich and Liebig were particularly involved. It is convenient to pick the idea up in the influential formulation by the plant physiologist F. F. Blackman (1905):

'When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor.'

Blackman called this an axiom, but others have been less careful and it has become known as Blackman's law of limiting factors. He followed this statement of the axiom with the following comment:

'I think one may fairly express surprise at the extent to which this principle has been overlooked by those who have proposed to work out the relation between a function and some *single* one of the various factors that control it.'

From this, and other explanations that follow it, it is clear that Blackman was chiefly concerned to warn against seeking to explain a process by variation in a factor which actually has little influence while neglecting another factor which has much greater influence. The axiom has, however, been wrenched from its setting and is now frequently believed to imply that only one factor can be limiting at a time. It is my purpose to show (later) that, whatever Blackman's intention, this belief is wrong.

Blackman discussed his ideas using a hypothetical example of the response of 'assimilation' (net photosynthetic) rate to concentration of carbon dioxide and light 'intensity' (flux). In a later article (Blackman & Smith, 1911) he used experimental measurements of the net rate of oxygen evolution of the water plants *Fontinalis antipyretica* and *Elodea canadensis* in relation to carbon dioxide concentration. In all these cases Blackman shows an upward sloping straight line at low supply values followed by a sharp transition to a horizontal line at higher supply. Later workers, for example Harder (1921) and Maberly (1985 and Fig. 5 of this article), have usually found that the shape is approximately hyperbolic, with an apparent asymptote. (One may suspect that had the factor been increased far enough then a negative response would have been found.) Some at least of the differences can be explained by the development of a thick boundary layer in Blackman's experiments which he made with unstirred solutions so that his study was dominated by the physical process of diffusion of carbon dioxide through water.

In what circumstances is a factor limiting? The answer is the same as it was for the recognition of a

nutrient: *if an increase in supply results in an increase in response then the factor is, in the conditions of the experiment, limiting.* In graphic terms this corresponds, again, to the condition that the line connecting two points on the response curve has a positive slope. The term 'limiting factor' is subject to the same need for qualification as was 'nutrient'. It seems to be rare though for those who study limiting factors to pursue the chase into the toxic range of supply.

In essence, the terms 'nutrient' and 'limiting factor' are both shorthand descriptions of the results of an experiment that should exist, but is often assumed. If this is accepted it follows that whenever a solute is a nutrient it is, *ipso facto*, a limiting factor. But temperature, for example, cannot in any useful way, be considered a nutrient. Light, which is usually treated as a limiting factor, might be considered as a nutrient if expressed as a photon flux density. Water itself may be in either category (or none when it is simply the medium).

### Rate-limiting and yield-limiting

Blackman considered rate-limiting processes, while Austin & Austin (Fig. 1) discussed yield-limiting ones. If the response were recorded as plant mass (on some common basis of area, individuals etc.) both would have the physical dimensions of  $MT^{-1}$ . So what is the relationship between them? The upper half of Fig. 2 shows a simple hypothetical case in which the instantaneous rate at the left is known for a relatively long period of time. The integral of this rate, over time, is the yield and is shown at the top right of Fig. 2. Discussions of the results of the instantaneous rate in the left hand curve might focus on a *particular time*, while discussions of yield based on the right hand curve should refer to a *specific elapsed time* over which the result, a rate still, gives the average. Those who measure yield often do not know what the time-course of the instantaneous rate has been, so their findings are of coarser texture than are those who make a complete growth analysis (Evans, 1972; Hunt, 1982). In compensation it is practicable for them to use a greater range of factors and treatment levels because they need make a measurement at only one time for each combination. Both approaches have their value.

A specific case is shown in the bottom half of Fig. 2, constructed from results recorded in Golterman *et al.* (1969). The data are the population density of *Scenedesmus obliquus* in flasks containing a fixed

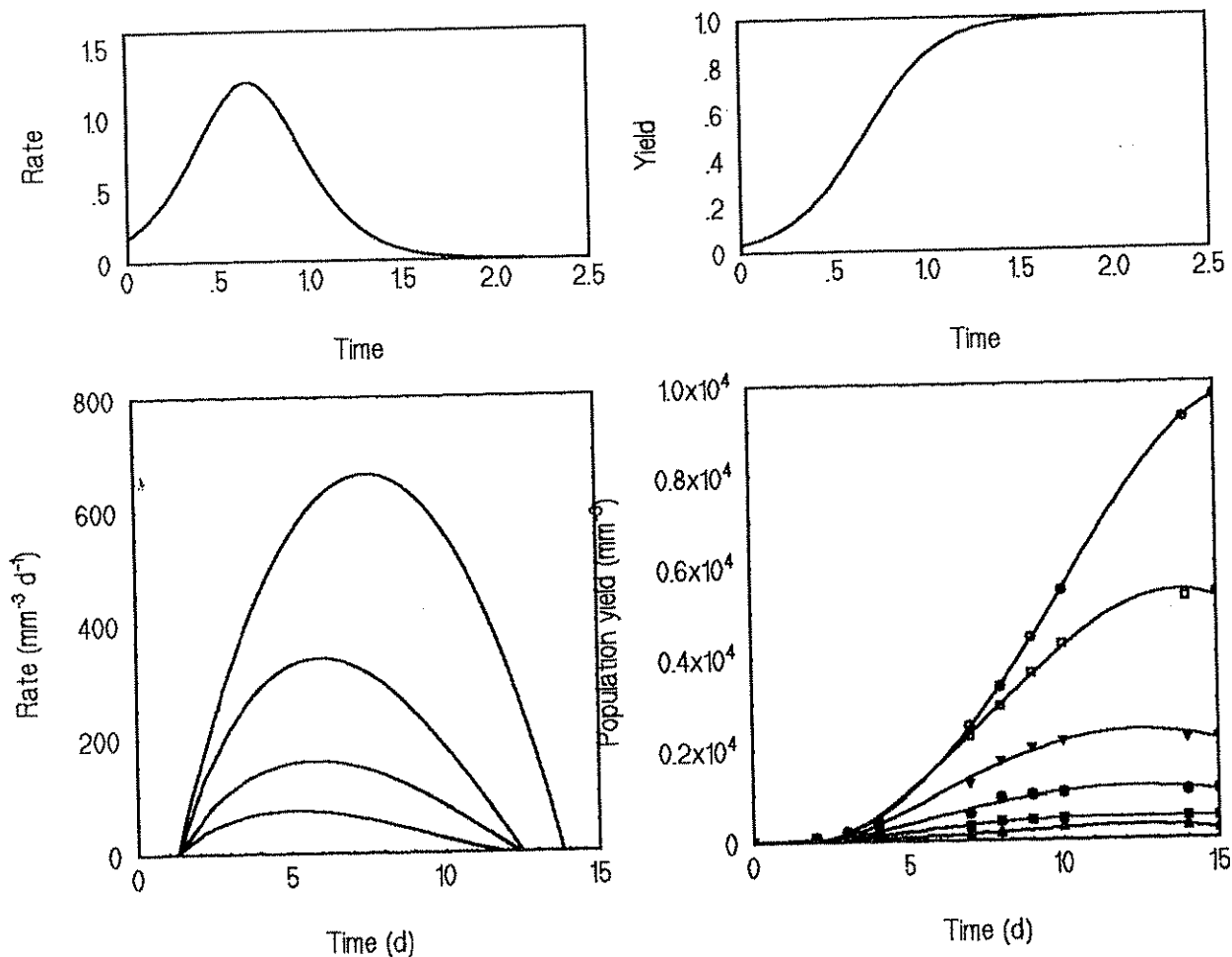


Fig. 2. Time course of instantaneous rates (left side) and yield – time averaged rate – (right side). The upper two graphs show a hypothetical case. The lower right are experimental measurements of the population density of *Scenedesmus* grown in flasks with initial concentrations of phosphate, from the lowest curve upwards, of 40, 50, 100, 200, 500 and 1000  $\mu\text{g l}^{-1}$ . The continuous curves are fitted fourth-order polynomials. These were differentiated (see text) to give the graph at the lower left. The curves there are, from the lowest upward, for phosphate concentrations of 100, 200, 500 and 1000  $\mu\text{g l}^{-1}$ . Results from Golterman, Bakels & Jakobs-Mögelin (1969).

volume of solution but differing initial concentrations of phosphate. Thus the 'supply' is a fixed amount of phosphate whose concentration declined during the experiment, and the 'response' is a yield. I have fitted polynomials to the curves and differentiated them to give the instantaneous rates at two times at the left of Fig. 3. Note that because a polynomial was used the small values of rates are suspect: a different degree of polynomial gives marked shifts at low rates. Fig. 3 also shows (part of) the yield response curve at the right, in this case for three times. If one assumes that the phosphate concentration after 8 days is related to the initial concentration then it is clear that phosphate concentration limited the instantaneous growth rate of *Scenedesmus* after 4 and 8 days in this experiment,

while the yield becomes more dependent on supply as time passes, perhaps being a response to the total supply.

#### More than one factor

Blackman's axiom has been interpreted as specifying that only one factor can be rate-limiting at once, and has been extended to suggest the same for yield-limiting factors. The results in Table 1 show that this latter conclusion, at least, is not true. The results are part of a wider survey by Pigott & Taylor (1964). In the case of *Alliaria petiolata*, addition of extra phosphate has caused an increased response, so phosphate is a nutri-

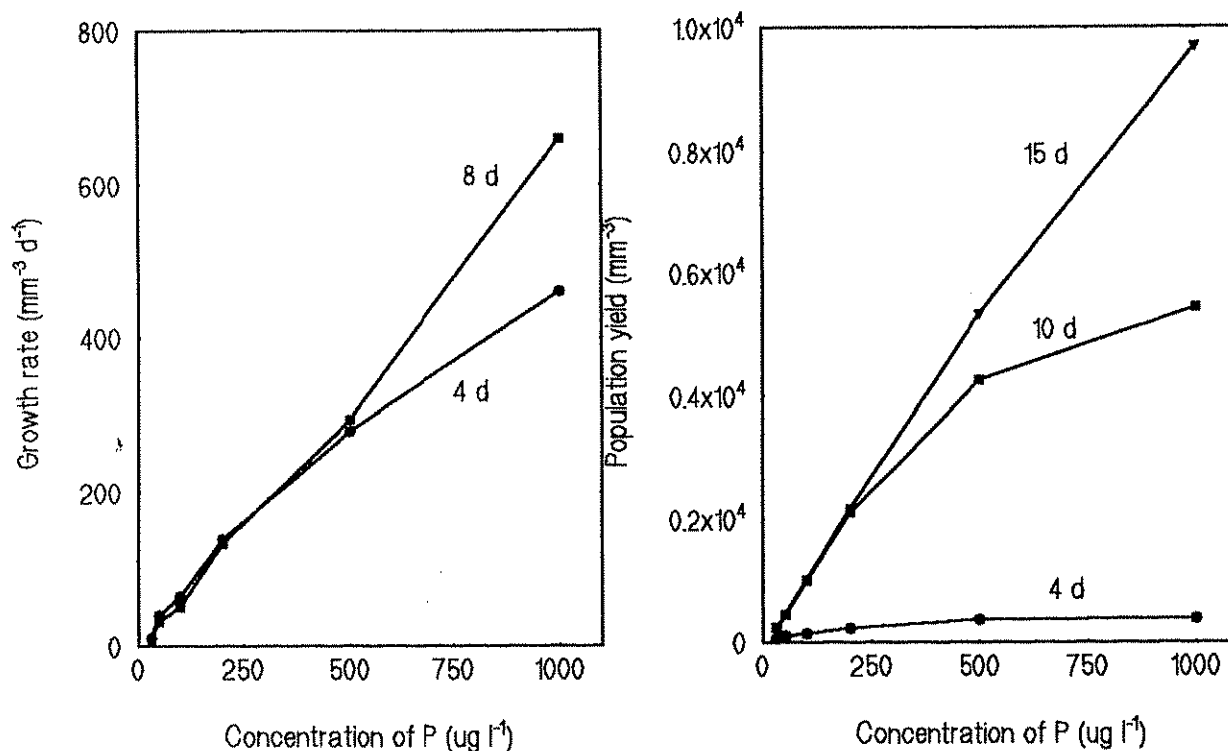


Fig. 3. Results from the lower half of Fig. 2 plotted as response curves. Instantaneous rates for two times at left; time-averaged rate (yield) for three times at right.

ent and a limiting factor in these circumstances. The response to additional nitrate on its own is also positive, though much smaller, so nitrate is also a nutrient and a limiting factor in these circumstances. The response to addition of both is almost what would be expected (it is well within the  $P = 0.05$  limits) by simple addition of independent effects. The graphic effect (Fig. 4, left) is of an almost flat plane connecting the four points and sloping upwards much more steeply on one axis than on the other. *The positive slope indicates a nutrient or limiting factor; the four coplanar points indicate that the effects are simply additive.*

*Galium aparine* (Table 1) shows different simple responses. By the same criteria, nitrate alone behaves as a nutrient and limiting factor, phosphate alone is not limiting. But together they produce a response much greater than a simple addition of the individual responses. This is an 'interaction' measurable by the difference between the expected response for simple addition and the actual response. In graphic form (Fig. 4, centre) this interaction appears as a bent sheet connecting the four points.

The results for *Mercurialis perennis* show a further possibility. By the same criteria again addition

of nitrate is actually toxic when no extra phosphate is added, while phosphate behaves as a nutrient and limiting factor when no extra nitrate is added. Together, however, they show a positive interaction. From another point of view, nitrate swings from acting as a toxin to acting as a nutrient when the supply of phosphate is increased. The bent sheet in Fig. 4, right, reflects this. *Positive slope on both axes together is, in general, the evidence that more than one factor can be limiting at once.*

In the medical world a positive interaction may be described as a synergism. There are powerful statistical techniques, based on the analysis of variance, for identifying interactions and for combining this with regression or its equivalent to quantify slopes on multiple axes. These methods are beyond the scope of this article though.

Extreme cases of interactions, such as the reversal in behaviour of nitrate when supplied to *Mercurialis perennis* (Fig. 4, right) are easily described in the nutrient/toxin terminology but there is no common way to describe such behaviour in terms of limiting factors.

The results in Table 1 and Fig. 4 are useful in laying bare the elementary possibilities and graphical

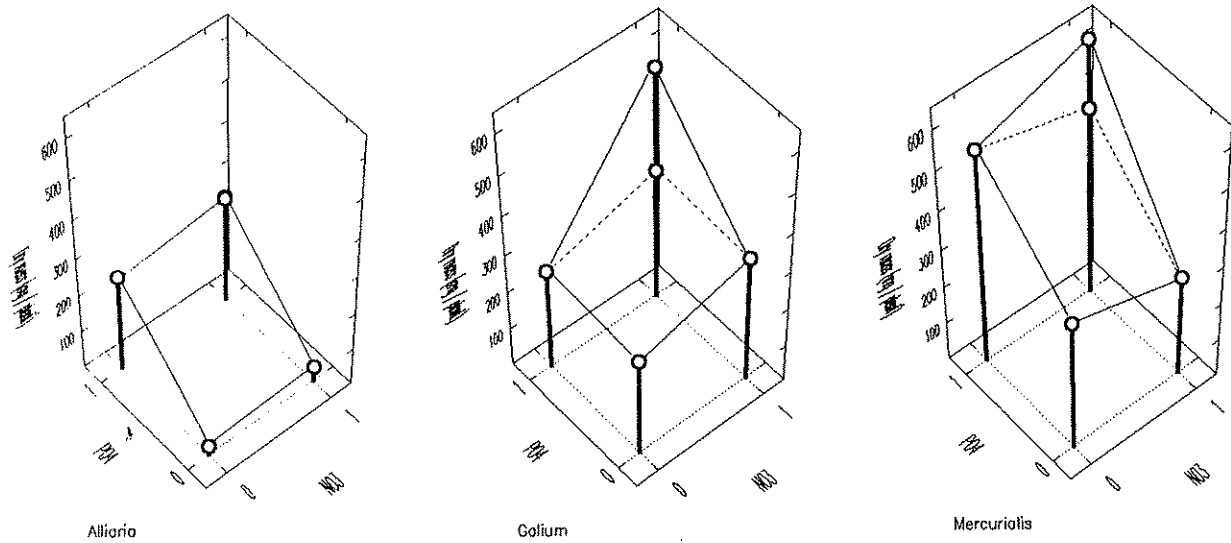


Fig. 4. Yield of three species (*Alliaria petiolata*, left; *Galium aparine*, centre; *Mercurialis perennis*, right) in response to factorial additions or no additions of nitrate and phosphate. Graphic plot of data in Table 1. See legend to that Table for details. Addition or no addition is shown arbitrarily as '1' and '0' respectively. Continuous lines show the measured results. Dashed lines show the result to be expected if there were no interaction between the two factors. Results from Pigott & Taylor (1965).

interpretations. Most experiments nowadays, however, use more than two treatment levels for each factor. An example is shown in Fig. 5 taken from Maberly (1985). This concerns the classic system considered by Blackman: the response of the water moss *Fontinalis antipyretica* to the supply of light and carbon dioxide. The graph may be considered in four quarters defined by values below (low) or above (high)  $\text{CO}_2$  concentration  $200 \mu\text{mol l}^{-1}$  and PFD below or above about  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . For low  $\text{CO}_2$  and high light the panels slope steeply up indicating that  $\text{CO}_2$  is limiting but light is not. The panels are almost rectangular (i.e. coplanar) so there is little interaction. The reciprocal is true for low PFD and high  $\text{CO}_2$ . At high supply of both factors the surface is nearly horizontal so neither factor is limiting. When both factors are low then the panels slope in both directions, so both factors are limiting, and the panels are trapezoidal, indicating interactions.

Results of two-factor experiments are frequently still plotted on a single X-Y plot, rather than the X1-X2 plot of Fig. 5. This is unsatisfactory because one factor must be arbitrarily chosen as the dominant one to appear as the X-axis while the other is represented as a series of lines. Making the second factor dominant may have a big effect on the appearance of the graph, though the data are the same. One point in particular is illustrated in the hypothetical case shown in Fig. 6. The factor F1 has been (arbitrarily) made dominant. The response to it, at level 1 of a second factor F2, is

shown in the lowest curve. Over the whole range of F1 the response increases as F1 increases so F1 is limiting. The same is true when F2 is at level 2 (a: the top curve). Furthermore, at all values of F1 the F2 level 2a curve is higher than the F2 level 1 curve i.e. an increase in F2 results in a higher response. Therefore F2 is limiting throughout as well as F1. Now consider an alternative response to F2 level (b: middle curve). Above the F1 supply of 0.7 the level 2b response is again higher than the level 1 response, so F2 is limiting (as well as F1). But for F1 less than 0.7 there is little difference between the F2 level 1 and level 2b responses, so F2 is not limiting in this range of F1. Where effects of this kind exist the use of the simple X-Y plot with the arbitrary choice of one of two factors for the X-axis, rather than the X1-X2 plot, can be very misleading.

#### Fitting the shape of response curves

There are numerous equations which can be persuaded, by a suitable choice of parameter values, to fit the general response curve that starts low, rises to a plateau, and then falls again. Fewer can be got to fit multifactor response curves (such as Fig. 5), but more can be got to fit just the initial rise and plateau. Thirty years ago the mechanics of fitting anything but a straight line were not simple and there was a premium on choosing an equation which could be transformed to a straight line.

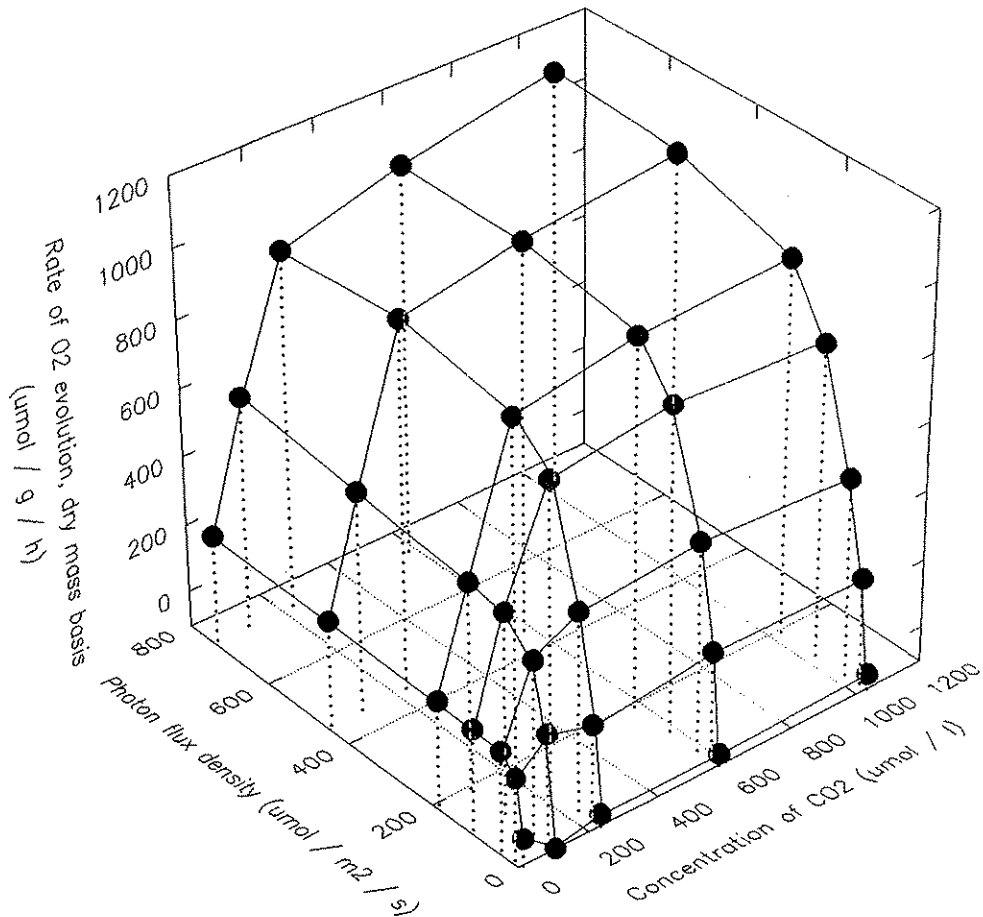


Fig. 5. Rate of  $O_2$  evolution on a plant dry mass basis, from plants of the water moss *Fontinalis antipyretica* collected in May, in response to the supply of  $CO_2$  and light. Results from Maberly (1985).

But there are now several methods of fitting almost any equation to a set of data (optimising the parameter values), and for obtaining estimates of precision, and these methods are fairly easily programmed for a desktop computer. One of the simplest and most robust fitting methods is the simplex of Nelder and Mead (1965), which requires no mathematics at all, though estimates of the precision of parameter values are more difficult. An excellent toolkit of programs (including this simplex) may be found in Press, Flannery, Teukolsky & Vetterling (1986).

Freed thus from the tyranny of the linear, what should guide one's choice of equation? There are at least three approaches in common use.

(1) The most general is to use a polynomial such as

$$z = b_2x^2 + b_1x + c_2y^2 + c_1y + dxy + a,$$

where  $z$  is the response variable,  $x$  and  $y$  are two supply variables (such as those in Fig. 5), and  $a$  to  $d$  are parameters (whose best fit values are to be found). The parameter  $d$  allows interaction between the variables  $x$  and  $y$ . If the main purpose is to get a close fit to a smooth set of data this technique (or some small complication of it) may be useful, provided that the dangers of extrapolation beyond the fitted data are remembered. The parameter values do not, in general, have any limnological or biological meaning, however, so this approach rarely improves understanding.

(2) A more informative approach is to choose an equation which has some physical meaning, such as the hyperbola, one formulation of which is

$$z = s/(1 + b/x),$$

where  $z$  and  $x$  are as before, and  $s$  and  $b$  are parameters. The underlying fact of this equation is that, for positive  $x$ , as  $x$  increases the response  $z$  rises toward an asymp-

Table 1. Yield (mg plant<sup>-1</sup>), after various times, of plants of three species grown in a woodland soil in which *Mercurialis perennis* grew. One pot was unamended, one had additional phosphate (+P), one had additional nitrate (+N), and a fourth pot had the same addition of both phosphate and nitrate. The marginal totals (+18, +234 etc.) show the effect of increasing the supply of one factor. The value in parentheses e.g. (275) is that expected if the effects of the two factors add in a simple way. The value in brackets e.g. [+6] shows the size of the interaction.

<i>Alliaria petiolata</i> (Hedge garlic) grown for 40 days			
Phosphate	Nitrate		
	No addition 0	Additional +N	
No addition, 0	23	41	+18
Additional, +P	257	(275) 281	
	+234	[+6]	
<i>Galium aparine</i> (Goosegrass, cleavers) grown for 55 days			
Phosphate	Nitrate		
	No addition 0	Additional +N	
No addition, 0	257	330	-27
Additional, +P	264	(337) 587	
	+7	[+250]	
<i>Mercurialis perennis</i> (Dog's mercury) grown for 88 days			
Phosphate	Nitrate		
	No addition 0	Additional +N	
No addition, 0	345	267	-78
Additional, +P	559	(481) 643	
	+214	[+162]	

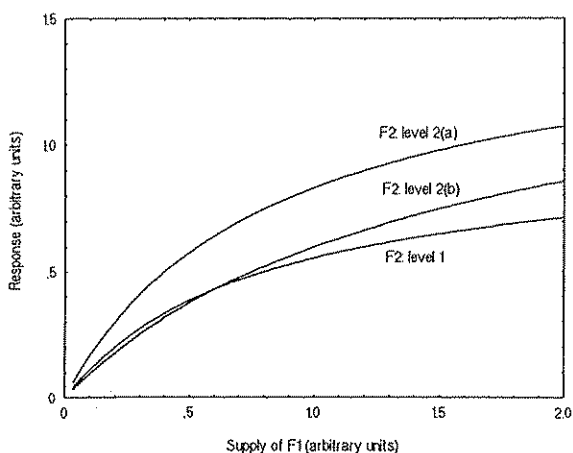


Fig. 6. Hypothetical response to supply of factor F1 for levels 1 and 2a, 2b of factor F2 (see text).

tote at  $z = s$ . This is close to the sort of assumptions that are often made when considering limiting factors, though it implies, strictly, that the factor  $x$  is limiting for all positive values of supply. For this equation the half-maximum, where  $z = s/2$ , is at  $x = b$ . Thus both parameters have easily understandable graphical interpretations. It is worth recognising that this equation is the same as that which emerges from specific assumptions about the processes of enzyme action, and which is then often called the Michaelis-Menten equation. Of course it does not (necessarily, or generally) follow that because a process shows hyperbolic 'kinetics' then it is a result of a simple enzyme reaction – though ignoring this has been the cause of much wasted effort. The general shape of the hyperbola is simple and often has systematic bias when fitted to response curve data. Modifications such as that of Smith (1936), developed by Talling (1957), to give

$$z = s/\sqrt{1 + (\beta x)^2},$$

increase the sharpness of the curvature and often improve the fit. The interpretation of  $\beta$  is different from that of  $b$  of course, but it does have a graphical and limnological meaning.

(3) The most fundamental approach is to start from specific assumptions about what is going on, then to derive equations that describe the overall response curve. As long as the underlying assumptions are not themselves specified as uninterpretable polynomial regressions then the parameters will have interpretable meanings.

### Comparison of factors

One sometimes sees a claim that factor A 'is more limiting' than factor B simply because the response to the arbitrarily chosen range of supply of A is greater than that for the equally arbitrary, and almost always different, range of supply of B. For this claim to have meaning the basis of comparison must be known. For chemicals it is possible to use a molar basis or, more dubiously, a mass basis. One might then point to the fact that, when the response curves for A and B are plotted with a common molar supply scale (and a common response scale) the slope of the A line is greater than that of the B line. In such conditions A might be said to be more limiting than B because, for a given change in supply, the response increases by more. But such clarity is rare, even when it might be achieved,



and it ignores interactions. A comparison of, for example, light and temperature, may be impossible.

### Optima

For a single factor response curve, such as those in Fig. 1, it is possible to point to the peak value and tempting to call it the optimum. This is a myopic use of optimum. The more interesting question concerns the multifactorial optimum, or (in the limit) the global optimum. A huge multifactorial experiment would, in principle, give the optimum (supposing there were only one) directly. A less demanding technique equates the problem to that of climbing a hill blindfold and assumes that the experiments can be made sequentially rather than simultaneously. One might make an initial experiment with factor A over a wide range to locate the peak. Then fix that value of the supply of A and combine it with a wide range of B to locate the peak for B. Then fix B and repeat the process for C then for D, E, F, and so on. If there were interactions among factors it would be necessary to iterate the process. The experiments could be made more efficient by using wide intervals for supply and then filling in only between the three that embrace the peak response. More complicated would be to employ the analogue of the blindfold person feeling around to find which way was steepest and then moving in that direction, changing the supply of all factors simultaneously. Nelder & Mead's (1965) simplex is a similar technique. These sequential, directed, techniques, however, may by chance climb a local small hill. In whichever direction they feel around, the surface slopes down, but the mountain (global) summit may be elsewhere and much higher. Such dangers are probably more theoretical than real in the present context.

One needs only to describe the processes to understand how rarely, if ever, has the global optimum been found let alone been demonstrably found. Suppose, however, that it has been found. What now is limiting the response? So far we have considered only external factors. There are internal factors too, many of which are not easily manipulated. Ultimately genetics and the laws of chemistry and physics must set the boundaries to the size of the response for a particular species.

### Conclusions

'Nutrient' and 'limiting factor' are like jargon: they speed communication amongst those who are expert. They may also mislead if used carelessly. It is possible, and probably the normal thing, for responses to be limited by more than one factor at once. Discussions of which factor is 'more' or 'most' limiting are sensible only if the factors have been expressed on a common basis.

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