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ESTIMATING N_2-FIXATION IN THE FIELD USING ^{15}N-LABELLED FERTILIZER: SOME PROBLEMS AND SOLUTIONS

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Summary—The ^{15}N-labelled fertilizer dilution technique provides a method of obtaining estimates of biological N_2-fixation in the field over the growing season. Field estimates of fixation obtained using peas, french beans, field beans and clover depended on the non-fixing control used. Differences in the N uptake patterns of the legume and control combinations, together with a decrease in the enrichment of plant available soil N with time, were major factors causing this dependency. A simple model of plant N accumulation at decreasing soil enrichment is presented, which explains these errors and allows a more rational choice of non-fixing control. The use of gypsum pelleted ^{15}N fertilizer, or any other treatment which leads to a more stable soil enrichment, reduces errors caused by mismatched N uptake patterns in the two crops.

INTRODUCTION

Methodology for the measurement of N_2-fixation by field crops has evolved over the past two decades. The classical approach by which an estimate of fixation was obtained by subtracting the N contained in a non-fixing crop from that of the legume crop is discredited by the required assumption that both crops obtain the same quantity of N from soil and fertilizer. More recently the rapid and sensitive acetylene reduction assay has been used (Hardy *et al.*, 1973). This method is of little use in obtaining an estimate of N_2-fixation over the growing season because of uncertain conversion ratios of C_2H_2 reduced to N_2 fixed, because of the large diurnal and longer term changes in rate of enzyme activity and because of difficulties in recovering and assaying total root nodule systems. A method based on the differential dilution in the plant of ^{15}N-labelled fertilizer by soil and fixed nitrogen (Fried and Broeshart, 1975; Fried and Middelboe, 1977) overcomes these difficulties and appears to offer a potentially accurate method for assessing seasonal N_2-fixation. But estimates obtained with this method vary depending on the non-fixing control crop chosen (Wagner and Zapata, 1982). The experiments described here were done to obtain an understanding of the reasons for these variations. Fixation estimates for several legume and control plant combinations were examined with a view to obtaining more accurate estimates of fixation and a more rational basis for the choice of non-fixing control crop.

MATERIALS AND METHODS

Experimental design

Field experiments were done at Woburn Farm near Rothamsted Experimental Station. The soil at this site is a mixture of sandy lower greensand and coarse loamy colluvium. The crops listed in Table 1 were hand sown between 2 and 8 April 1979 in 7 × 2 m

Table 1. Nitrogen yield, ^{15}N enrichment and derived values[1] for several crops at final harvest (Mean of 4 replicate plots ± SD)

Crop	N level (kg NO_3^--N ha^{-1})	Atom% ^{15}N excess	N uptake (kg ha^{-1})	% Fertilizer uptake	Soil *A*-value
Oilseed rape	30	0.456 ± 0.020	72.5 ± 5.6	29.5 ± 0.09	223 ± 11.3
	30 pel[2]	0.496 ± 0.018	78.4 ± 4.4	34.2 ± 2.2	203 ± 8.9
Barley	30	0.333 ± 0.011	50.0 ± 4.0	14.6 ± 1.7	317 ± 12
	30	0.362 ± 0.031	50.1 ± 6.2	16.5 ± 3.2	297 ± 29
Grass	30	0.552 ± 0.065	36.3 ± 1.3	17.2 ± 1.6	190 ± 14
	30 pel	0.514 ± 0.058	42.1 ± 5.2	18.7 ± 2.1	195 ± 10
Grass	150	0.372 ± 0.017	140 ± 9.8	42.8 ± 2.0	175 ± 16
French bean	30	0.223 ± 0.013	168 ± 7.2	33.3 ± 2.6	
	30 pel	0.253 ± 0.017	171 ± 7.4	37.3 ± 1.6	
Pea	30	0.197 ± 0.004	133 ± 8.0	22.7 ± 0.9	
	30	0.216 ± 0.010	131 ± 5.2	27.3 ± 1.9	
Red clover	30	0.190 ± 0.005	95.1 ± 3.5	15.6 ± 0.4	
	30 pel	0.247 ± 0.012	100 ± 3.9	21.6 ± 1.1	
Field bean	30	0.188 ± 0.015	274 ± 16.4	45.2 ± 5.7	
	30 pel	0.196 ± 0.017	275 ± 16.1	46.5 ± 7.6	

[1]The values for percentage fertilizer uptake and soil N pool are the means of separate determinations for the replicate plots and are not identical to those which can be calculated from the mean N uptake and atom% excess values given in the table.
[2]pel = gypsum pellets.

plots arranged randomly in four replicate blocks. Peas (*Pisum sativum* cv. Kelvedon Wonder), french beans (*Phaseolus vulgaris* cv. Cascade) and field beans (*Vicia faba*, cv. Minden) were planted in rows 30 cm apart at 70 seeds m^{-1}. Barley (*Hordeum sativum* cv. Victor) was planted in 5 cm rows to give about 1000 seeds m^{-2}. Perennial ryegrass (*Lolium perenne* cv. Caprice), red clover (*Trifolium pratense* cv. Hungaropoly) and oilseed rape (*Brassica rapa* cv. Eurora) were broadcast and raked into the seedbed at 4, 2 and 2 g m^{-2} respectively. A compound fertilizer (0:14:28) was applied to all plots before sowing at 336 kg ha^{-1}. Isotopically-labelled fertilizer was applied, as either a solution of $K^{15}NO_3$ or as gypsum pelleted $K^{15}NO_3$, to 1.25 m^2 sub-plots (legumes) or to a 1.25 × 7 m strip (controls) within each plot. The gypsum pelleted fertilizer was turned into the seedbed to a depth of 20 cm. Rates of application were equivalent to 30 kg N ha^{-1} (3.835 atom% excess) or 150 kg N ha^{-1} (0.768 atom% excess) and the same quantities of unlabelled N were applied to the area surrounding the subplots. Alternate strips 0.75 × 0.3 m were taken at successive harvests leaving 0.3 m strips as a guard area between harvests.

Nitrogen determinations

Crop N was determined from dry weight and Kjeldahl N content at each harvest. ^{15}N enrichments were determined in subsamples of the Kjeldahl digest by emission spectrometry (Lloyd-Jones *et al.*, 1974). Six core samples (30 × 200 mm) were taken from the grass and oilseed rape plots 10 days before the first harvest and at each subsequent harvest for soluble N determinations. Soil samples from each plot were bulked, mixed and sieved. Subsamples (100 g) were shaken with 100 ml 2 M KCl and the concentrations of NO_3^- and NH_4^+ in the supernatant were determined colorimetrically after filtration (Analysis of crops and fertilisers, 1978). Isotopic enrichments were determined spectrophotometrically in subsamples prepared by a Conway microdiffusion procedure modified using Devados alloy to include both NO_3^--N and NH_4^+-N (Conway, 1939).

Xylem exudate was collected from the oilseed rape in 1 ml plastic syringe barrels connected to the cut plant stems by short lengths of butyl rubber tubing. Exudate from eight plants was pooled for Kjeldahl digestion. Salicylic acid and sodium thiosulphate were added to prevent the loss of NO_3^--N (Analysis of crops and fertilisers, 1978). Isotopic enrichments were determined as for the plant samples. Exudate could not be collected at all harvests because of dry soil conditions or senescent plants.

Preparation of gypsum pellets

Surgical plaster (98% gypsum) was found to be an inexpensive and convenient pelleting material. Commercially available building plaster was found to be unsuitable because of its high pH. Pellets for each subplot or strip were prepared separately from aliquots of $K^{15}NO_3$ stock solution. The setting time for gypsum decreases when the mixing water contains dissolved salts (to *ca.* 15 s with 10% KNO_3) and the procedure described here was evolved so that mixing and pelleting could be carried out as rapidly as possible. Plaster (200 g) was weighed into 12 × 20 cm polythene bags and 100 ml of stock solution was added. The bag was agitated briskly for 15 s to obtain an even mixture which was spread into 4 mm wide by 5 mm deep grooves in a ridged rubber mat. After 2 min the plaster strips so formed were released by bending the mat and broken into 1–2 cm lengths.

RESULTS

The pea crop attained a maximum N content 120 days after sowing while french and field beans reached a maximum about 10 days later (Fig. 1). The N content of the clover crop was still increasing at final harvest. The N uptake pattern of oilseed rape was similar to that of pea but grass receiving 150 kg N ha^{-1} took up considerably more N than the other crops early in the season. Nitrogen limitation induced early maturity in barley receiving 30 kg N ha^{-1} and sequential harvesting of this crop was discontinued after 30 August.

The ^{15}N enrichment of KCl-extractable mineral N beneath control plots receiving non-pelleted $K^{15}NO_3$ are shown in Figs 2, 3 and 4. Soil enrichment declined exponentially for about 100 days after N application. Thereafter the relative availability of fertilizer and soil N stabilized to give, in the case of grass plots receiving 30 kg N ha^{-1} (Fig. 2) a continuing but much slower rate of decrease ($\log_e$ atom% ^{15}N excess $day^{-1} = 0.005$). A similar stabilisation is suggested by the later values from oilseed rape and grass 150 kg N

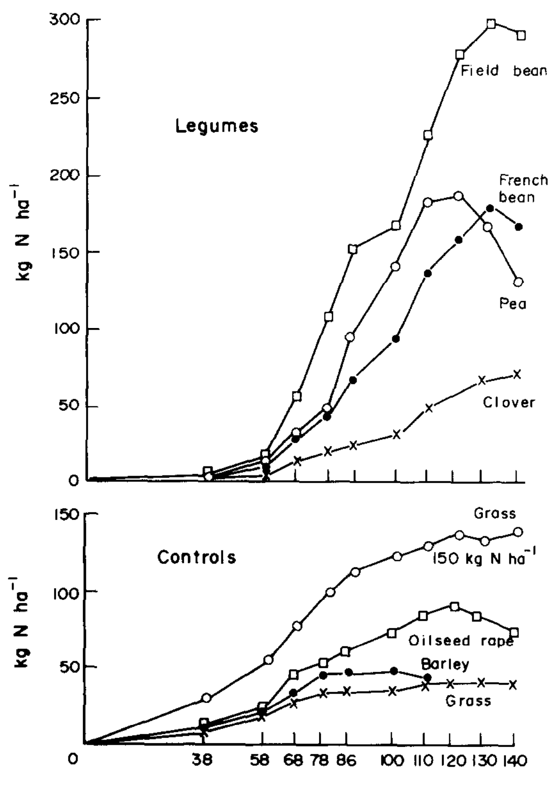


Fig. 1. Nitrogen uptake patterns for several legumes and non-fixing control crops. Crops were planted between April 2 and 8. Final harvest was on 18 September.

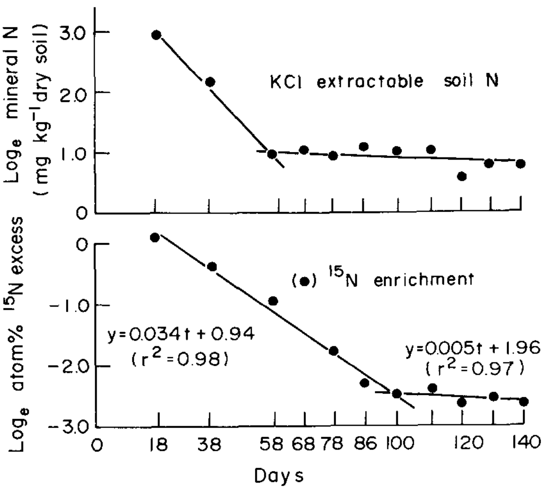


Fig. 2. Amount and ^{15}N enrichment of KCl extractable soil mineral N beneath grass plots receiving 30 kg N ha^{-1} (3.835 atom% ^{15}N excess).

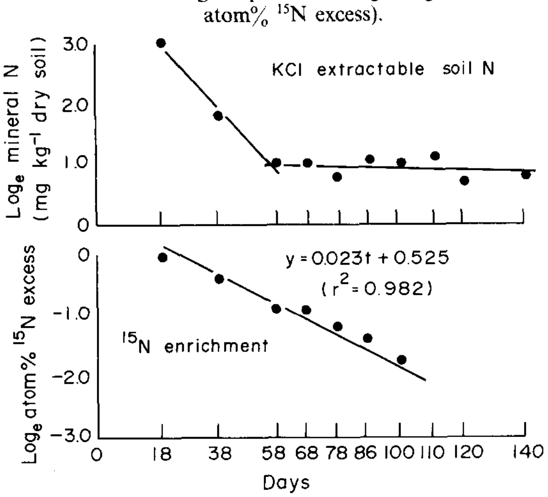


Fig. 3. Amount and ^{15}N enrichment of KCl extractable soil mineral N beneath oilseed rape plots receiving 30 kg N ha^{-1} (3.835 atom% excess).

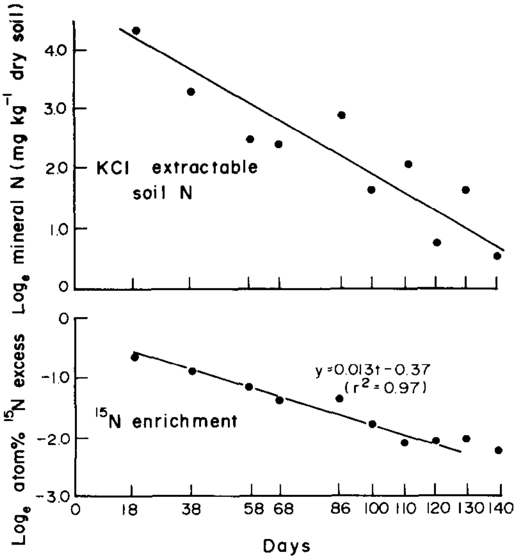


Fig. 4. Amount and ^{15}N enrichment of KCl extractable soil mineral N beneath grass plots receiving 150 kg N ha^{-1} (0.768 atom% excess).

Table 2. Estimates of N_2-fixation by four legume species calculated by difference and by isotope dilution relative to several controls (kg N ha^{-1} season^{-1} ± SE)

Control	N level (kg NO_3^--N ha^{-1})	Red clover: Isotope dilution	Red clover: Difference	Field bean: Isotope dilution	Field bean: Difference	Pea: Isotope dilution	Pea: Difference	French bean: Isotope dilution	French bean: Difference
Grass	150	63.0	(19.3)	181	(198)	86.5	(57.2)	100	(92.5)
Grass	30	60.8	58.8	174	238	83.1	96.7	95	131
Grass	30 pel[1]	51.4	57.9	170	233	69.6	88.9	87.0	129
Rape	30	55.6	22.6	160	201	75.6	60.5	83.7	95.5
Rape	30 pel	49.7	21.6	166	196	67.4	52.6	84.1	92.6
Mean[2]		56.1	40.2	170.3	217.1	76.4	74.7	90.0	112.0
SE		2.06	7.85	2.87	8.04	2.96	8.03	2.58	7.80
Barley	30	40.9	45.1	117	224	54.2	82.9	52.4	118
Barley	30 pel	29.4	49.9	123	225	41.7	80.9	49.0	121

[1]pel = gypsum pellets.
[2]Neither the parenthesized difference values nor the barley data have been included in the mean.

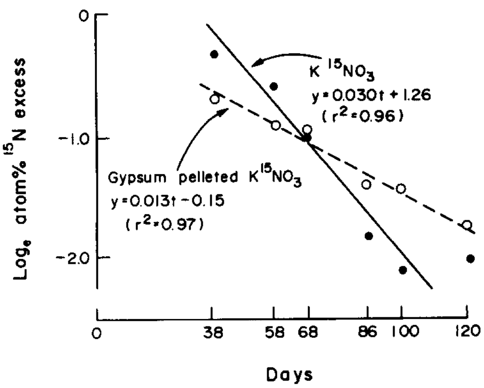


Fig. 5. Isotopic enrichment of xylem exudate from oilseed rape receiving gypsum pelleted or unpelleted N at 30 kg ha^{-1} (3.835 atom% excess).

plots (Figs 3 and 4) but the effect is less clear. The rate of isotopic decline beneath grass was reduced at the higher N level with a value of 0.013 at 150 kg N ha^{-1} compared with 0.034 at 30 kg N ha^{-1}. Total soluble N also showed a biphasic decline at the lower N levels (Figs 2 and 3) with a well defined rate change at about 60 days.

Isotopic enrichments of xylem exudate from oilseed rape are shown in Fig. 5. The ratio of $^{15}N/^{14}N$ passing up the plant stem declined exponentially with time as the soil enrichment fell. The regression gradient was reduced from 0.030 to 0.013 by gypsum pelleting the fertilizer N.

The total N content of crops from plots receiving gypsum pelleted fertilizer were not significantly different from those receiving KNO_3 as a solution (Table 1). Isotopic enrichment, with the exception of barley, and percentage fertilizer uptake for all crops was higher when N was applied as gypsum pellets. Soil N pool estimates are not presented for the legume crop because they are not needed for the calculation of fixation (see Discussion).

Table 2 shows seasonal N_2-fixation by the legume crops relative to the various controls estimated by isotope dilution and by the classical subtraction method. The estimates have, with the exception of the grass control at 150 kg N ha^{-1}, been calculated from legume and control pairs receiving the same fertilizer treatment. Such a pairing was not possible for the grass given 150 kg N because this amount of N would have depressed fixation by the legume. The difference based estimates relative to these high N grass plots (parenthesized in Table 2) have been calculated after subtracting from the total N in the grass the amount derived from fertilizer (42.8% of 150 kg, Table 1).

DISCUSSION

Calculations and assumptions

The conceptual and mathematical methods normally presented for the calculation of N_2-fixation from ^{15}N fertilizer dilution data are derived from the A-concept of Fried and Dean (1952) and operate by considering differences in the apparent soil N pool size (A-value) in the fixing and non-fixing control crops. The A-value is a measure of the soil nitrogen pool in fertilizer equivalents, normally calculated as:

$$A \text{ soil} = [(100 - \%\text{Ndff})/(\%\text{Ndff})] \times \text{amount of fertilizer applied} \quad (1)$$

where A soil = size of the soil N pool in fertilizer equivalents, and %Ndff = % of plant N derived from fertilizer.

Fixation is normally calculated as:

$$N_2\text{-fixed} = [(\% \text{ fertilizer uptake by fixing crop})/100] \times (A \text{ soil fixing crop} - A \text{ soil non-fixing crop}) \quad (2)$$

Provided that the same quantities of labelled fertilizer are added to both crops absolute amounts need not be known. The relevant terms in equation (2) cancel (Fried and Middleboe, 1977; Rennie *et al.*, 1976) to give:

$$N_2\text{-fixed} = [1 - (\text{atom\% } ^{15}\text{N excess in fixing crop})/(\text{atom\% } ^{15}\text{N excess non-fixing crop})] \times (\text{total N in fixing crop}) \quad (3)$$

The calculation described here is based on simple isotope dilution. The soil A-value is an estimate of the N available to the plant over the growing season calculated from the dilution of added ^{15}N by soil N. It is assumed that the plant cannot discriminate between ^{15}N and ^{14}N so that the enrichment in the soil and the control crop is the same, hence:

$$\begin{aligned} &\text{atom\% } ^{15}\text{N excess in non-fixing crop} \\ &= \text{atom\% } ^{15}\text{N excess in soil} \\ &= 100 \times (^{15}\text{N excess in soil})/(\text{total available N in soil}) \\ &= (Rf \times Nf)/(A \text{ soil non-fixing crop} + Nf) \end{aligned}$$

where

Nf = rate of fertilizer application,

and

Rf = atom% ^{15}N excess in fertilizer.

Therefore

$$A \text{ soil non-fixing crop} = [(Rf \times Nf)/(\text{atom\% } ^{15}\text{N excess in non-fixing crop})] - Nf \quad (4)$$

The apparent soil N pool based on the enrichment of the fixing crop (A soil fixing crop, equation 2) need not be known. The amount of N fixed is the total in the legume less that derived from soil and fertilizer. In both methods of calculation the plant is assumed to take up fertilizer and soil N in proportion to the amount available so that the proportion of soil N taken up is the same as the proportion of fertilizer N taken up. Hence:

$$N_2\text{-fixed} = \text{total N in fixing crop} - \frac{\%\text{fertilizer uptake by fixing crop}}{100} \times \left(A \text{ soil non-fixing crop} + \text{fertilizer added}\right) \quad (5)$$

The equation in this form is mathematically identical to the normally presented equation (2) and it is applicable to data from experiments where the same or different quantities of labelled fertilizer are used on the fixing and non-fixing crops. The derivations of equations (4) and (5) are presented elsewhere (Witty, 1983).

An *A*-value based estimate of fixation is not, as has been stated (Fried and Broeshart, 1975), an estimate of the amount of N fixed by a crop. It is rather an estimate of the amount of fixed N contained in the crop. This becomes evident when equation (5) is rearranged as:

$$\text{Total N in fixing crop} = \text{N}_2\text{-fixed} + \frac{\%\text{fertilizer uptake by fixing crop}}{100} \times \left(A \text{ soil non-fixing crop} + \text{fertilizer added}\right)$$

Although there is an efficiency factor for the uptake of soil and fertilizer N (% fertilizer uptake by fixing crop/100) there is no such factor for fixed N. It is assumed in the equation that all the fixed N is taken up so that the coefficient for the uptake of fixed N is unity (i.e. 100% uptake). In practice this is unlikely. Even if the entire root system could be recovered at harvest at least some of the fixed N would have been lost to the soil by leakage and by root and nodule senescence during the life of the crop. Only part of this N would be taken up again by the roots.

Thus the *A*-value based estimate is a measure of the fixed N accumulated in the harvested portion of the crop, rather than an estimate of the total amount of fixed N entering the soil–plant system. The difference between these two values depends on the proportion of fixed N lost from the crop and the efficiency with which this is recovered.

Fried and Broeshart (1975) stated that the only assumption inherent in the *A*-value method itself is that a plant confronted with two sources of nutrient will take up nutrient in proportion to the amounts available. But a number of other assumptions are made in the application of this technique to the field measurement of N_2-fixation. Fried and Broeshart (1975) advocate a larger fertilizer N addition to the control crop, the assumption presumably being that the added N has no effect on the N cycling processes in the soil. The priming effect by which added N increases the availability of soil N is, however, controversial (see Broeshart, 1974, Hauck and Bremner, 1976). A further assumption, if the legume and control are to see the same soil N pool, is that either fertilizer distribution is entirely even with depth or that the rooting patterns of the legume and control are the same.

Effects of a decline in soil enrichment

Although problems associated with the priming effect and fertilizer distribution can be avoided partly by careful choice of control and the use of the same N level on both crops, the data presented here demonstrate that considerable errors can result from a changing soil enrichment and different N uptake patterns of the legume and control crops. That the enrichment of mineral N in the soil does fall with time after the application of ^{15}N is shown by the KCl extract data presented in Figs 2, 3 and 4. Analysis of xylem exudate from oilseed rape (Fig. 5) confirms that this is a real decline in the enrichment of N available to the plant. The decrease in ratio is presumably accounted for by a loss of plant available ^{15}N due to uptake, leaching and immobilization and by the continuing release of soluble ^{14}N by mineralization.

The mechanism by which this change in soil enrichment affects *A*-value based estimates of N_2-fixation can be examined by considering a simple model in which the crops accumulate soil N which is decreasing in isotopic enrichment. The crop N accumulation curves shown in Fig. 6 are based on the logistic curve;

$$\text{Crop N} = N_\infty/[1 + e^{-K(t-l)}] \tag{6}$$

where

N_∞ = maximum crop N,
K = crop growth rate constant,
t = time in days,
and l = time to half maximum N content.

Decreases in soil enrichment over the initial period when most crop N is taken up fit reasonably to an exponential ($r^2 > 0.97$, Figs 2, 3 and 4) and the soil enrichments shown in Fig. 7 have been modelled as:

$$\text{atom\% } ^{15}\text{N in soil} = a + b e^{-Dt} \tag{7}$$

where

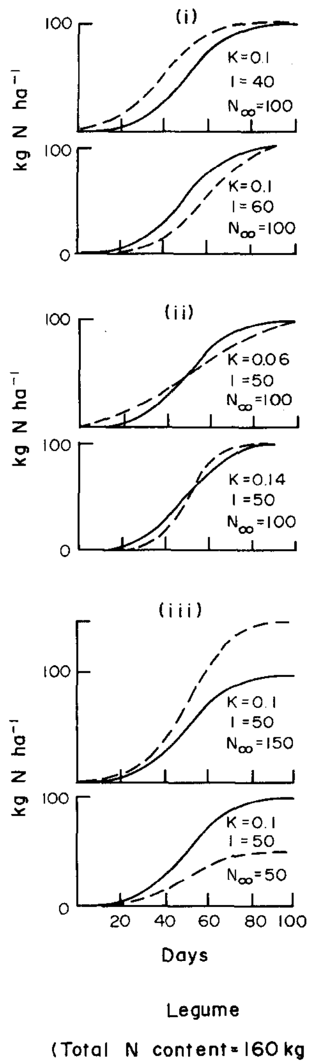
a = enrichment at time infinity,
b = atom% excess time zero,
t = time in days,
and D = decline constant.

The enrichment at time infinity (a in equation 7) has been taken as natural abundance. D values of 0.01 and 0.05 (Fig. 6) were chosen to span the experimentally determined decline rates (Figs 2, 3 and 4).

Integration of N increments at decreasing isotopic enrichments with time give the atom% excess values shown at the side of Fig. 6. These values represent the enrichment which would be found in a crop whose N accumulation pattern is shown as a dotted line in the figure at final harvest. The percent fertilizer uptake and soil N pool (*A*-value plus added fertilizer N) were calculated from these enrichments assuming that 5 kg N ha^{-1} had been added to the soil at 10 atom% ^{15}N excess. The solid line in each figure represents the uptake of soil N by the standard legume crop and the values shown at the end of the table have been calculated from this curve assuming that the legume contains 160 kg N ha^{-1}, half of which comes from fixation. The fixation estimate shown in the first column opposite Fig. 6i is the value which would be obtained when a crop with the N uptake pattern shown in this figure is used as a control for the standard legume and the isotopic enrichment in the soil is declining at a rate $D = 0.01$. Fixation was estimated as in equation (5), in this case:

$$\text{Fixation} = 160 - (49.1/100) \times 148$$

The subtractive term is the percent fertilizer uptake by the legume at $D = 0.01$ (from the legume data at the bottom of the table) multiplied by the soil N pool estimate from the control at the same D-value.



	^{15}N decline constant, D 0.01	0.05
(i)		
Atom% excess	0.336	0.178
Soil N pool	148	280
N_2-fixed	87	108
Atom% excess	0.279	0.074
Soil N pool	179	675
N_2-fixed	72	35
(ii)		
Atom % excess	0.310	0.147
Soil N pool	161	340
N_2-fixed	81	99
Atom% excess	0.305	0.099
Soil N pool	164	505
N_2-fixed	79	66
(iii)		
Atom% excess	0.307	0.116
Soil N pool	163	431
N_2-fixed	80	80
Atom% excess	0.307	0.116
Soil N pool	163	431
N_2-fixed	80	80
Legume (Total N content = 160 kg ha^{-1})		
Atom% excess	0.153	0.058
% Fert. uptake	49.1	18.5
(Soil N pool	326	862)

Fig. 6. Modelled N uptake curves for several controls (dotted lines) relative to a standard legume (solid line). The enrichment which would occur in these controls at final harvest, at two rates of decline in soil enrichment, have been calculated from equations (6) and (7). The soil N pool is the soil *A*-value plus the amount of fertilizer added. The correct estimate of fixation is 80 kg N ha^{-1}. See text for full explanation.

Figure 6 shows that the estimates of fixation are not affected greatly by N uptake pattern when the rate of change in soil enrichment is small. With a *D*-value of 0.01 estimates range from 72 to 87 kg ha^{-1} compared with the correct value of 80 kg ha^{-1}. As the rate of ^{15}N decline increases however the errors incurred by mismatching controls becomes larger, and with a *D* value of 0.05, fixation estimates range from 35 to 108 kg N ha^{-1} depending on the control chosen.

The way in which these errors occur is evident from the data in Fig. 6i. Although the legume and control take the same total quantity of N from the soil the control shown at the top Fig. 6i takes up N earlier and hence at a higher enrichment than the legume. The soil N pool, calculated from the enrichment of the control, is thus smaller than that appropriate to the legume and fixation is overestimated. Conversely,

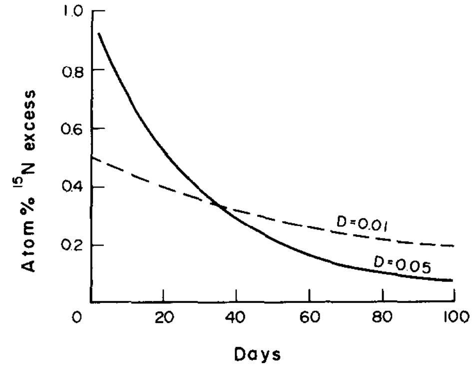


Fig. 7. Modelled decline of ^{15}N enrichment in soil. Curves are based on equation (7) with initial enrichments of 0.5 and 1 atom% excess to simulate the effect of gypsum pelleted ^{15}N fertilizer.

if the control takes up N later than the legume, an underestimate of fixation occurs. At the higher decline rate in this model estimates of fixation actually become negative when the control l value (equation 6) exceeds 65 days.

Figure 6iii represents the only circumstance where the A-value method operates accurately against a background of declining soil enrichment. That is when the growth constants K and l (equation 6) are the same for the legume and control, so that the relationship between the N-uptake rate of the two crops remains constant with time. In this case the total N uptake by the control makes no difference to the size of apparent soil N-pool.

Interpretation of field estimates of fixation

Differences in the ^{15}N based estimates of fixation presented in Table 2 can be interpreted with respect to declining soil enrichments and the mechanisms proposed in the model. If the N uptake constants cannot be determined for the crops being tested they must at least start to increase in N content together and reach their maximum at the same time. Errors in the estimates of fixation will be reduced by treatments which lead to a more stable soil enrichment.

Grass receiving 150 kg N ha^{-1} took up N earlier than the other crops at a consequently higher enrichment. Therefore the apparent soil N pool (175 kg ha^{-1}, Table 1) is low relative to the other controls (mean 237 kg ha^{-1}) and fixation estimates relative to this control are high (Table 2). The decrease in soil N pool associated with an increased N fertilizer addition is the reverse of that which would be given by a priming effect (see Hauck and Bremner, 1976). Although such an effect may have occurred it is masked in this case by the changed N uptake pattern of the crop.

The nitrogen deficient barley was a very poor control both because of its early maturity and harvest and because of its delayed and limited N uptake (Fig. 1). The soil N pool calculated from the enrichment in the barley is high and fixation estimates using this control are consequently low. The data are parenthesized in Table 2 and are included only to demonstrate the principle involved.

For field beans, french beans and peas the most appropriate control is oilseed rape (Fig. 1). For these crops the best estimates of fixation are those relative to this control obtained with gypsum pelleted ^{15}N fertilizer. Grass was an inappropriate control for these legumes but the error incurred by mismatched N uptake patterns depends on the rate of change in soil enrichment; the values relative to grass 30 kg N ha^{-1} are thus considerably closer to the values obtained with oilseed rape when the applied fertilizer N was gypsum pelleted. With an ideal control estimates of fixation are independent of the rate of change in soil enrichment (Fig. 6iii). Thus differences in fixation estimates caused by treatments which alter the rate of change in soil enrichment are themselves indicative of a mismatching between the two crops. Grass receiving 30 kg N ha^{-1} was probably the best control for red clover and the best estimate of fixation is again that obtained using pelleted ^{15}N.

Wagner and Zapata (1982) used the A-value method to measure N_2-fixation by soyabeans and field beans relative to several controls. These authors note that a suitable reference crop should utilize soil and fertilizer N in the same proportion as the legume crop. In order to check this proportionality they applied ammonium sulphate fertilizer labelled with ^{15}N and ^{35}S. They concluded that all the controls were equally satisfactory in giving values of fixation with an error of estimates of less than 10%. In their field bean experiments the soil A-values at final harvest ranged from 434 kg ha^{-1} with oil radish, to 705 kg N ha^{-1} using sudangrass. This gave estimates of fixation by field beans ranging from 125 to 144 kg N ha^{-1}. Fertilizer uptake by this crop, of 6.1%, was extremely low. The size of the soil A-value therefore made little difference to the estimates of fixation since only 6.1% of the soil N was taken up. The soyabean data presented by these authors in the same paper shows a 21.4% N fertilizer uptake at an early harvest and an 18.8% uptake at final harvest. Fixation estimates ranged, at the first harvest, from 4 to 27 kg N ha^{-1} and at the second harvest from 40 to 59 kg N ha^{-1} depending on the control used.

Rooting pattern and fertilizer distribution

Differences in the rooting pattern of the crops are indicated by different fertilizer recoveries (Table 1) and uneven fertilizer distribution is suggested by differences in the apparent rate of decline in the enrichment of soil N. Estimates of this parameter so far presented have been determined by direct measurement on soil extracts and xylem sap. These values can also be obtained by fitting experimentally determined N accumulation data to equations (6) and (7). The results for grass receiving non-pelleted fertilizer are shown, as an example, in Fig. 8. The rates of decline in $^{15}N/^{14}N$ ratios measured in various ways are summarized in Table 3. Both xylem exudate and curve fitting data from oilseed rape show that the decline in the ratio of fertilizer to soil N is more than halved by gypsum pelleting the fertilizer. A similar, though smaller, effect can be seen in the values derived by curve fitting for the lower N grass plots. The disparity in estimates of decline based on soil extracts and those obtained by curve fitting for the grass 30 kg N plots is probably due to uneven vertical

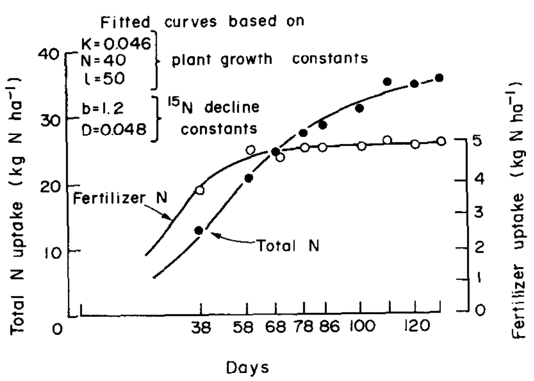


Fig. 8. Modelled curves fitted to experimentally determined data from grass plots receiving 30 kg N ha^{-1} as a solution of $K^{15}NO_3$ at 3.835 atom% excess.

Table 3. Rates of decline ($\log_e$ atom% ^{15}N excess day^{-1}) in ^{15}N enrichment of soil mineral N beneath grass and oilseed rape plots calculated in several ways. See text for explanation

Crop	N treatment ($kg\ NO_3^-$-$N\ ha^{-1}$)	KCl extract	Estimate based on xylem exudate	Curve fitting
Grass	30	0.034	—	0.048
Grass	30 pel[1]	—	—	0.037
Grass	150	0.013	—	0.020
Oilseed rape	30	0.023	0.030	0.023
Oilseed rape	30 pel[1]	—	0.013	0.011

[1]pel = gypsum pellets.

distribution of N and a soil sampling procedure which extracted N from below the root zone of the grass. The fertilizer uptake for oilseed rape was higher than that for grass (Table 1) suggesting that the difference in decline values obtained by curve fitting for these two crops are due to the more deeply rooted oilseed rape extracting fertilizer N which had moved down the soil profile below the root zone of the grass.

Difference based estimates of fixation

Fixation can be estimated by subtracting the N content of a non-fixing control from that of the legume only when both crops obtain the same quantity of N from soil and fertilizer. Although the quantity of soil N taken up is not directly proportional to ^{15}N labelled fertilizer recovered the two are at least related. Thus difference based estimates approach those obtained using the isotope dilution method (Table 2) when the percent fertilizer uptake of the legume and control (Table 1) are similar. The difference based estimate of 58.8 kg N fixed by red clover (15.6% fertilizer uptake, Table 1) relative to grass receiving 30 kg N ha^{-1} (17.2% fertilizer uptake, Table 1) is close to the value of 60.1 estimated by isotope dilution (Table 2). If the same calculation is made using oilseed rape (29.5% fertilizer uptake, Table 1) as a control for this crop an erroneously low estimate of 20.1 kg N fixed is obtained. Similar effects can be found throughout the table.

Field estimates of fixation based on the difference in N content of a legume and control combination having the same percentage fertilizer uptake could not be reliably obtained because the relative fertilizer uptake of the two crops will vary from season to season. The method does however provide a useful comparison for isotope dilution based estimates when similar uptakes do, by chance, occur.

Conclusions

The major problem in applying the ^{15}N fertilizer dilution method to the measurement of N_2-fixation is the determination of a soil N pool (A-value) which is appropriate to the fixing crop. Other values required for the estimate (total N content and % fertilizer uptake of the fixing crop, equation 5) are determined directly; the soil N pool value can be obtained only indirectly using a non-fixing control crop.

The accuracy of the method depends on the choice of an appropriate non-fixing control. The ideal legume-control combination should have (1) similar rooting patterns and (2) similar N uptake profiles, specifically the same K and l values as defined in equation (6). Even the relatively small differences in uptake pattern shown in Fig. 6 lead to a 50% error when the rate of change in soil enrichment is high. The best approach in initial experiments may be the use of a range of controls so that the researcher can gain some feeling for which is most appropriate to his own legume, conditions and experimental objectives. A perfect matching is seldom possible but the magnitude of error incurred will depend upon the rate of decline in soil enrichment. Nitrogen fertilizer treatments, such as direct incorporation of ^{15}N-labelled organic matter (Hauck, 1973), the addition of the fertilizer together with an available carbon source (Legg and Sloger, 1975) or the use of slow release ^{15}N fertilizer formulations, which lead to a more stable soil enrichment, will give a more accurate isotope dilution based estimate of N_2-fixation.

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