PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Biological Nitrogen Fixation and Grassland Production in the United Kingdom

D. W. Cowling

Phil. Trans. R. Soc. Lond. B 1982 **296**, 397-404
doi: 10.1098/rstb.1982.0015

Phil. Trans. R. Soc. Lond. B **296**, 397–404 (1982)
Printed in Great Britain

Biological nitrogen fixation and grassland production in the United Kingdom

BY D. W. COWLING†

The Grassland Research Institute, Hurley, Maidenhead, Berks. SL6 5LR, U.K.

Grassland occupies almost two-thirds of the agricultural land in the United Kingdom; it has a varied botanical composition and sometimes includes legumes that fix N_2. Estimates of the amount of N fixed have been made in field experiments; for mixtures of white clover with grass it varies from 74 to 280 kg ha^{-1} annually, and for crops of red clover and lucerne it is at the higher end of that range. The contribution from white clover and other legumes sown with grass declines markedly with increasing addition of fertilizer N. In agricultural practice, white clover is rarely a major constituent of swards, reflecting a 30-fold increase in the use of fertilizer N during the last 35 years, and it is estimated that the average amount of N fixed annually is 11 kg ha^{-1}. The areas sown to red clover and lucerne are small and are declining. Experimental data suggest that fixation by free-living organisms is much less than fixation by legumes. In practice the contribution from fixation may be improved by more careful culture of white clover in swards, and by an increased use of leguminous forage crops to provide conserved feeds.

1. INTRODUCTION

Biological N_2 fixation has been an important source of N in most agricultural systems throughout the world. Cato (234–149 B.C.), in his agricultural treatise *De re rustica*, acknowledged the value of leguminous plants in raising soil fertility, either directly or through the provision of manure derived from their use as feed (Fussell 1971). Early in this century, grassland production in the United Kingdom was much improved by encouraging the growth of white clover (*Trifolium repens* L.) through the use of phosphatic fertilizers (Davies 1952). More recently the nature of our grassland has changed with less reseeding and a marked increase in the use of fertilizer N. During the early 1940s, the average amount of N added annually in fertilizer was 4 kg ha^{-1}; now it is 128 kg ha^{-1} (Church & Leech 1980). With grassland occupying 7.2 Mha, almost two-thirds of the agricultural area of the United Kingdom, these additions have a substantial impact on the nitrogen cycle.

As with many processes in the nitrogen cycle, there is difficulty in quantifying biological N_2 fixation. In this paper, the measurements that have been made in field experiments are reviewed and used as a basis for estimating the role of biological N_2 fixation in our grassland production.

2. EXPERIMENTAL MEASUREMENT OF BIOLOGICAL N_2 FIXATION

While precise estimates of fixation may be obtained under controlled conditions of culture, it is also important to assess the rates under field conditions. A common method is to establish a nitrogen balance. The following equation, based on Greenland (1977), relates a change in

† Died 13 July 1981.

the amount of N in a given mass of soil, ΔN, to the difference between the several inputs and outputs of N, during a specified period:

$$\Delta N = (F+S+R+M+D)-(C+L+V+E), \qquad (1)$$

where F = N fixed biologically; S = N transferred from greater soil depth; R = N added through rainfall; M = N added through fertilizers, manures and seeds; D = N added through the deposition of particulates and dust, and through the absorption of gases; C = N removed in crops and present in above-ground parts of the plant; L = N leached to groundwater; V = N lost to the atmosphere through volatilization (as NH_3) and denitrification (as N_2 and N_2O); E = N lost in run-off and erosion. Biological N_2 fixation, F, or indeed any other single component, may be determined from measurements of all the others. However, all the components are rarely measured together in one study. Many are difficult to measure precisely, though others may be absent or negligible or are already known.

The use of ^{15}N has allowed improvement in techniques for measuring processes in the nitrogen cycle (Hauck & Bremner 1976), but it has to be emphasized that discrimination may strictly be made between only two sources, or components, in the balance. Recently, ^{15}N has been used in conjunction with the concept of 'A' value (Fried & Broeshart 1975) to estimate symbiotic fixation of N_2 by legumes grown alone or with grasses (Deibert *et al.* 1979; Haystead & Lowe 1977; Phillips & Bennett 1978). The authors noted have emphasized the assumptions that have to be made; at present they are not generally accepted. Thus, it remains to be confirmed that the supply of N from symbiotic fixation does not significantly alter the relative uptake of N derived from soil organic matter and from fertilizer.

The acetylene-reduction technique provides a useful means of measuring biological N_2 fixation (Halliday & Pate 1976), but it has been little used in grassland studies in the United Kingdom. It has the disadvantage of requiring many successive assays if fixation is measured over long periods, and the ratio of $N_2:C_2H_2$ reduced, as well as its variation, needs to be known (Masterson & Murphy 1976). Also, the technique will not discriminate between N_2 fixation by symbiotic and free-living organisms.

3. Measured rates of N_2 fixation

(*a*) *Symbiotic* N_2 *fixation*

The *Rhizobium*–legume symbiosis represents a source of N in grassland in the United Kingdom mainly through the presence of white clover, but there are also contributions from other legumes, principally red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.). The amount of N fixed symbiotically through white clover has been estimated in a number of field experiments (e.g. those by Brockman & Wolton (1963), Chestnutt (1970, 1972), Cowling (1961, 1966), Herriott & Wells (1960), Reid & Castle (1965) and Reid (1970, 1972)). The experiments included two kinds of swards, namely grass alone and grass with white clover, that were treated in a similar manner. The yield and N content of the crops harvested in successive cuts were measured separately for the grass and clover components of the mixed sward. Often fertilizer N was added at a similar range of rates to each kind of sward. Nitrogen fixation, and the influence on it of fertilizer N, was estimated from the difference between the amounts of N in the crops from the two swards given similar additions of N.

In using this method, it is tacitly assumed that components other than fixation included in the balace equation (1) are similar with the two swards. Within reasonable limits, this may be true for gains of N through rainfall and deposition, and for losses through run-off. It may not be true for losses through leaching, volatilization and denitrification, nor for changes in content of soil N. No account is taken of differences in the content of N in the unharvested parts of the plants, e.g. the stubble, roots and stolons. With increasing duration of the experiment, this N may turn over and contribute to increased plant uptake as well as to changes in soil N. It is unfortunate that data for soil N have been reported for few of the above experiments. However, with swards of cocksfoot (*Dactylis glomerata* L.) sown alone or together

Table 1. The amount of N fixed symbiotically by white clover (*Trifolium repens* L.)

(The estimate is based on the N content of the harvested parts of: (*a*) the clover, the 'direct contribution', and (*b*) the companion grass, additional to that measured in grass sown alone, i.e. the 'indirect contribution'.)

N fixed annually kg ha^{-1}				
direct	indirect	total	notes	reference
86	71	157	3 years, 2 grass species	Herriott & Wells (1960)
119	66	185	6 years	Cowling (1961)
–	–	185	3 years	Holliday & Wilman (1962)
128	39	167	1 year	Brockman & Wolton (1963)
129	55	184	4 years	Armitage & Templeman (1964)
58	45	103	3 years, 2 grass species	Reid & Castle (1965)
133	38	171	3 years, 8 grass species	Cowling & Lockyer (1967)
109	59	168	3 years	Reid (1970)
127	73	200	5 years, 8 grass species	Chestnutt (1972)
64	59	123	3 years	Reid (1972)
61	29	90	4 years	Munro & Davies (1974)

with white clover, the mean annual increments of N in the soil, including roots, were 70 and 108 kg ha^{-1} respectively, while annual crop removals were 30 and 205 kg ha^{-1} (Clement & Williams 1967). In this experiment, and probably in others, symbiotic fixation is underestimated if no account is taken of the changes in soil N.

Data from several experiments (table 1) show the amount of N fixed annually by white clover in swards given no fertilizer N; exceptionally, the amount has reached 280 kg ha^{-1}. Apart from the N in the harvested clover (the 'direct contribution' of symbiotically fixed N), the yield of grass in the mixed sward, and its content of N, are almost always greater than those of grass growing alone. The mechanism of this benefit (the 'indirect contribution' of symbiotically fixed N) is not clearly known; it is thought to be the death of clover tissue, especially root, nodule and stolon, with the subsequent mineralization of the N that this contains. The variation among sites in the total amount of N fixed is high; it is also high between years at a single site, e.g. 74 to 280 kg N ha^{-1} (Cowling 1961). This variation reflects mainly the sensitivity of the growth of white clover to the supply of available soil water (Low & Armitage 1959). The decline with time from sowing, in the yield of white clover in mixture with grasses, and in the amount of N fixed, is less with irrigation than without (Reid & Castle 1965; Stiles & Williams 1965).

Data from some of the experiments with white clover provide a comparison of the relative contribution of N from symbiotic fixation and from fertilizer to the yields of dry matter (figure 1*a*) and of N (figure 1*b*) in the harvested crops. There is, of course, variation among experiments

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in the yields of grass and clover as well as in the slope of the regression lines relating them to the rate of added fertilizer N. The amount of clover, and of N fixed, declines markedly with added N (figure 2).

The presence of white clover may account for as much as 75 % of the gross yield of dry matter from a mixed sward given no fertilizer N, especially on soils low in available soil N. To produce

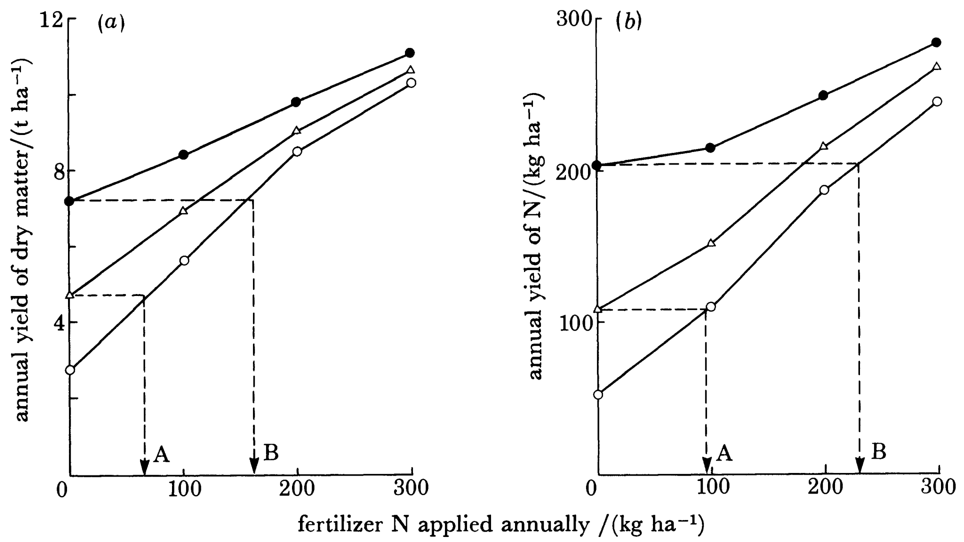

FIGURE 1. The relation of the annual yields of (*a*) dry matter and (*b*) N, of grass and grass–clover swards to the amount of fertilizer N applied. ●, Grass–clover; △, grass in grass–clover; ○, grass alone. A and B are, respectively, the fertilizer equivalents of the indirect and the gross contribution of clover in a grass–clover sward. (Based on Brockman & Wolton (1963), Chestnutt (1970, 1972), Cowling (1961, 1966), Herriott & Wells (1960), Reid & Castle (1965) and Reid (1970, 1972).)

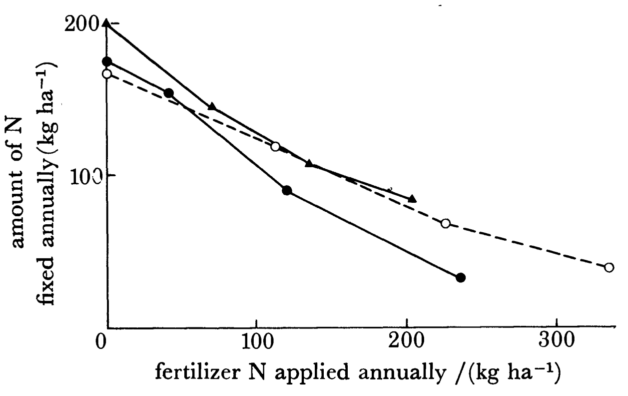

FIGURE 2. The relation of the amount of N fixed symbiotically by white clover, grown with grass, to the amount of fertilizer N applied. ●, Cowling (1961); ○, Reid (1970); ▲, Chestnutt (1972).

a similar yield, grass alone requires fertilizer N to be added at between 150 and 200 kg ha^{-1} annually (Chestnutt & Lowe 1970). The N content (percentage) of clover is invariably higher than that of well fertilized grass, so that the value of clover is higher on the basis of yield of N than on the basis of yield of dry matter (see figure 1*a*, *b*).

Nitrogen fixation in a mixture of white clover and grass has also been estimated in the field by using the acetylene reduction technique (Halliday & Pate 1976). The amount fixed annually was 268 kg ha^{-1}, somewhat higher than in the experiments reviewed above. This method will have included N that was fixed and that remained in unharvested parts of the plants and in the soil.

Bell & Nutman (1971) and Nutman (1976) assessed fixation by the *Rhizobium*–lucerne symbiosis by using a 'difference method' similar to that described for the white clover experiments. Nitrogen fixation annually by lucerne ranged from 90 to 342 kg ha^{-1} (mean 225) in established crops given adequate supplies of potassium and phosphorus (Nutman 1976). Other estimates of the amount of N fixed annually by lucerne are: 263 kg ha^{-1} (Williams & Cooke 1972); 230 kg ha^{-1} and, in association with grass, 221 kg ha^{-1} (Davies 1964). An estimate of N_2 fixation by red clover of 219 kg ha^{-1} (Williams & Cooke 1972) is supported by yields for this species, alone or in mixture, of about 10 t ha^{-1}, containing 2.6% N (Frame 1976).

It is not proposed to discuss here in detail the effect on forage production of the recirculation, through grazing, of N derived from biological fixation. There is evidence for a significant positive effect where fertilizer is the main source of N; without such addition the evidence is equivocal (Green & Cowling 1960). Thus, where clover is the main source of N, there is often little difference in yield, or in the N content of the crop, between swards that are grazed and those that are harvested by cutting, or that receive no dung and urine (Jackson & Williams 1979; Wheeler 1958).

(*b*) *Fixation of* N_2 *by free-living organisms*

The acetylene-reduction technique was used to measure fixation in core samples of soils under grassland from 56 locations in England and Wales (Lockyer & Cowling 1977). It was concluded that fixation rarely exceeds 5 kg N ha^{-1} annually and is due mainly to blue-green algae. This rate agrees with measurements from grassland of Scotland (Stewart *et al.* 1978). Also, in common with other studies (Stewart *et al.* 1975), fixation was reduced with addition of fertilizer N. In vegetation composed of coarse grasses and perennial dicotyledons, fixation accounted for much of an annual increment of soil N of 39 kg ha^{-1} and was associated with organisms living in the rhizosphere of the dicotyledons (Witty *et al.* 1977).

Because of large additions of fertilizer N to grassland, fixation by free-living organisms is unlikely to be of great importance. However, the increases in soil N that are sometimes measured under grass are not easily explained. For example, over a 3 year period there was an annual gain of 70 kg N ha^{-1} in the soil (0–75 mm) under a sward of grass alone given no fertilizer N, and where rainfall added 12 kg N ha^{-1} (Clement & Williams 1967).

4. Estimated fixation of N_2 in grassland

The content of clover (species of *Trifolium* and other legumes) in grassland, based on percentage ground cover, is recorded in a recent farm survey (Forbes *et al.* 1980). The grassland was representative, in terms of age, utilization and rate of added fertilizer N, of enclosed grassland in England and Wales and, for practical purposes, in the United Kingdom. The proportions of the surveyed area of two ages of grassland found in four categories, based on clover content (0, 1–5, 5–15, 15–25%), are shown in table 2. More than a third of the grassland is devoid of clover, and only a quarter has clover representing more than 5% of the ground cover. If it is assumed, not unreasonably, that these contents of clover are similar to those in the harvested crops, and that they are inversely related to annual yields of dry matter for the four clover categories, of 7, 6, 5 and 4 t ha^{-1} (Green & Baker 1981), then the amounts of clover would be 0, 180, 500 and 800 kg ha^{-1} respectively. With a N content of 4%, the amounts

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

represent direct contributions through fixation of 0, 7, 20 and 32 kg N ha^{-1}. The ratio of the indirect to the direct contribution of fixed N is about 0.5 (figure 1*b*), so that the total amounts fixed would be 0, 10, 30 and 48 kg ha^{-1} annually for grassland in the four categories. Combining these data with the areas of grassland in the United Kingdom, apportioned on the basis of the survey to the four clover categories, gives an estimate of the total amount of N fixed (table 3). Including the limited area of lucerne that is grown, and assuming no addition through free-living organisms, the annual contribution of N from biological fixation in grassland is about 82 kt.

Table 2. Proportion of grassland (percentage) with various contents of clover†

content of clover (percentage ground cover) ...	0	1–5	5–15	15–25
class of grassland				
under 5 years old	37.5	36.1	19.8	6.6
5 years old and over	38.5	42.3	17.3	1.9

† Includes species of *Trifolium* and other legumes.

Table 3. Estimates of the annual amount of N fixed symbiotically by clover† in grassland, and by lucerne, in the United Kingdom

	area/kha	N fixed / kg ha^{-1}	total N fixed / t
grassland, under 5 years old			
no clover	721	0	0
clover, 1–5%	694	10	6940
clover, 5–15%	380	30	11400
clover, 15–25%	127	48	6096
grassland, 5 years old and over			
no clover	1998	0	0
clover, 1–5%	2196	10	21960
clover, 5–15%	898	30	26940
clover, 15–25%	99	48	4752
lucerne	16	237	3792
total	**7129**	—	**81880**

† Includes species of *Trifolium* and other legumes.

5. Discussion

The mean amount of N estimated to be added annually through biological fixation, 11 kg ha^{-1}, is small compared with the amount apparently fixed by clover in the experiments described in §3*a*. This amount may be estimated by difference as 94 kg ha^{-1} at current rates of fertilizer N use (figure 1*b*). The experimental swards differed from those found in practice in having clover well developed before fertilizer N was added; their clover content was much higher. It may be useful to adopt a similar procedure in practice, and also to develop effective techniques to introduce clover into established swards. Other measures to encourage the retention of clover in swards are less easy to design. Removing N from the system in crops cut for conservation, rather than recycling it by grazing, may increase the contribution from fixation. In most experiments the swards have been harvested by cutting; where grazing treatments have been included the clover contribution is often reduced (Green & Cowling 1960; Wheeler 1958).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The contribution from fixation may be increased by closer attention to the nutritional requirements of the *Rhizobium*–legume symbiosis, and by breeding legumes for improved symbiosis and greater persistency. It is important to achieve the benefit of such increases in mixed swards given fertilizer N, otherwise its use at the current average rate may only give yields similar to the potential of swards with a good complement of clover and given no fertilizer N. On the other hand, practices that encourage the retention of clover in mixed swards given large amounts of fertilizer N may result in the clover's being largely dependent on that source at the expense of fixation (Clement & Jones 1977). It is worth noting that the inclusion of legumes with grass results in improved nutritive value for the ruminant, feed intake as well as the utilization of digested energy being greater than with grass alone (Thomson 1979).

The other main avenue for greater exploitation of biological fixation in practice is through an increased role of leguminous forage plants, especially red clover and lucerne, for the provision of conserved feed. About 40 % of the total crop from our grassland is conserved, yet the areas currently devoted to red clover and lucerne are only about 40 and 16 kha, respectively.

I wish to thank Dr L. H. P. Jones and Mr J. O. Green for useful discussions and comments on the manuscript.

References (Cowling)

Armitage, E. R. & Templeman, W. G. 1964 Response of grassland to nitrogenous fertilizer in the west of England. *J. Br. Grassld Soc.* **19**, 291–297.

Bell, F. & Nutman, P. S. 1971 Experiments on nitrogen fixation by nodulated lucerne. *Pl. Soil*, special volume, pp. 231–264.

Brockman, J. S. & Wolton, K. M. 1963 The use of nitrogen on grass/white clover swards. *J. Br. Grassld Soc.* **18**, 7–13.

Chestnutt, D. M. B. 1970 Interaction of grass species grown alone and in mixtures to the presence of white clover. *Rec. agric. Res.* (*N. Ir.*) **18**, 143–154.

Chestnutt, D. M. B. 1972 The effect of white clover and applied nitrogen on the nitrogen content of various grass/clover mixtures. *J. Br. Grassld Soc.* **27**, 211–216.

Chestnutt, D. M. B. & Lowe, J. 1970 Agronomy of white clover/grass swards: A review. In *White clover research* (Occasional Symposium no. 6, British Grassland Society) (ed. J. Lowe), pp. 191–213. Hurley: British Grassland Society.

Church, B. M. & Leech, P. K. 1980 *Survey of fertilizer practice*, Duplicated report no. SS/SAFC/39. London: Ministry of Agriculture, Fisheries & Food.

Clement, C. R. & Jones, L. H. P. 1977 Growth and nitrogen fixation by clover grown at different concentrations of nitrate in flowing solution culture. *J. Sci. Fd Agric.* **28**, 870–871.

Clement, C. R. & Williams, T. E. 1967 Leys and soil organic matter. *J. agric. Sci., Camb.* **69**, 133–138.

Cowling, D. W. 1961 The effect of white clover and nitrogenous fertilizer on the production of a sward. *J. Br. Grassld Soc.* **16**, 281–290.

Cowling D. W. 1966 The response of grass swards to nitrogenous fertilizer. In *Proc. 10th Int. Grassld Congr. Helsinki, 1966*, pp. 204–209.

Cowling, D. W. & Lockyer, D. R. 1967 A comparison of the reaction of different grass species to fertilizer nitrogen and to growth with white clover. *J. Br. Grassld Soc.* **22**, 53–61.

Davies, W. 1952 *The grass crop*. London: Spon.

Davies, W. E. 1964 The yields and composition of lucerne, grass and clover under different systems of management. *J. Br. Grassld Soc.* **19**, 263–270.

Deibert, E. J., Bijeriego, M. & Olson, R. A. 1979 Utilization of ^{15}N fertilizer by nodulating and non-nodulating soybean isolines. *Agron. J.* **71**, 717–723.

Frame, J. 1976 The potential of tetraploid red clover and its role in the United Kingdom. *J. Br. Grassld Soc.* **31**, 139–152.

Forbes, T. T., Dibb, C., Green, J. O., Hopkins, A. & Peel, S. 1980 *Factors affecting the productivity of permanent grassland.* Hurley: The Grassland Research Institute.

Fried, M. & Broeshart, H. 1975 An independent measurement of the amount of nitrogen fixed by a legume crop. *Pl. Soil* **43**, 707–711.

Fussell, G. E. 1971 *Crop nutrition: science and practice before Liebig*. Kansas: Coronada Press.

Green, J. O. & Baker, R. D. 1981 Classification, distribution and productivity of U.K. grasslands. In *Grassland in the British economy* (ed. J. L. Jollans), pp. 237–247. Reading: Centre for Agricultural Strategy.
Green, J. O. & Cowling, D. W. 1960 The nitrogen nutrition of grassland. In *Proc. 8th In. Grassld Congr. Reading, 1960*, pp. 126–129.
Greenland, D. J. 1977 Contribution of micro-organisms to the nitrogen status of tropical soils. In *Biological nitrogen fixation in farming systems of the tropics* (ed. A. Ayanaba & P. J. Dart), pp. 13–25. Chichester: Wiley-Interscience.
Halliday, J. & Pate, J. S. 1976 The acetylene reduction assay as a means of studying nitrogen fixation in white clover under sward and laboratory conditions. *J. Br. Grassld Soc.* **31**, 29–35.
Hauck, R. D. & Bremner, J. M. 1976 Use of tracers for soil and fertilizer nitrogen research. *Adv. Agronomy* **28**, 219–266.
Haystead, A. & Lowe, A. G. 1977 Nitrogen fixation by white clover in hill pasture. *J. Br. Grassld Soc.* **32**, 57–63.
Herriott, J. B. D. & Wells, D. 1960 Clover nitrogen and sward productivity. *J. Br. Grassld Soc.* **15**, 63–69.
Holliday, R. & Wilman, D. 1962 The effect of white clover, fertilizer nitrogen and simulated animal residues on yield of grassland herbage. *J. Br. Grassld Soc.* **17**, 206–213.
Jackson, M. V. & Williams, T. E. 1979 Response of grass swards to fertilizer nitrogen under cutting or grazing. *J. agric. Sci., Camb.* **92**, 549–562.
Lockyer, D. R. & Cowling, D. W. 1977 Non-symbiotic nitrogen fixation in some soils of England and Wales. *J. Br. Grassld Soc.* **32**, 7–11.
Low, A. J. & Armitage, E. R. 1959 Irrigation of grassland. *J. agric. Sci., Camb.* **52**, 256–262.
Masterson, C. L. & Murphy, P. M. 1976 Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field. In *Symbiotic nitrogen fixation in plants* (ed. P. S. Nutman), pp. 299–316. Cambridge University Press.
Munro, J. M. M. & Davies, D. A. 1974 Potential pasture production in the uplands of Wales. *J. Br. Grassld Soc.* **29**, 213–223.
Nutman, P. S. 1976 IBP field experiments on nitrogen fixation by nodulated legumes. In *Symbiotic nitrogen fixation in plants* (ed. P. S. Nutman), pp. 211–237. Cambridge University Press.
Phillips, D. A. & Bennett, J. P. 1978 Measuring symbiotic nitrogen fixation in rangeland plots of *Trifolium subterraneum* L. and *Bromus mollis* L. *Agron. J.* **70**, 671–674.
Reid, D. 1970 The effect of a wide range of nitrogen application rates on the yields from a perennial ryegrass sward with and without white clover. *J. agric. Sci., Camb.* **74**, 227–240.
Reid, D. 1972 The effects of the long-term application of a wide range of nitrogen rates on the yields from perennial ryegrass swards with and without white clover. *J. agric. Sci., Camb.* **79**, 291–301.
Reid, D. & Castle, M. E. 1965 The response of grass/clover and pure-grass leys to irrigation and fertilizer nitrogen treatment. *J. agric. Sci., Camb.* **65**, 109–119.
Stewart, W. D. P., Haystead, A. & Dharmawardene, M. W. N. 1975 Nitrogen assimilation and metabolism in blue-green algae. In *Nitrogen fixation by free-living microorganisms* (ed. W. D. P. Stewart), pp. 129–158. Cambridge University Press.
Stewart, W. D. P., Sampaio, M. J., Isichei, A. O. & Sylvester-Bradley, R. 1978 Nitrogen fixation by soil algae of temperate and tropical soils. In *Limitations and potentials for biological nitrogen fixation in the tropics* (ed. J. Dobereiner, R. H. Burris, A. H. Hollaender, A. A. Franco, C. A. Neyra & D. B. Scott), pp. 41–63. New York: Plenum.
Stiles, W. & Williams, T. E. 1965 The response of a ryegrass/white clover sward to various irrigation regimes. *J. agric. Sci., Camb.* **65**, 351–364.
Thomson, D. J. 1979 Effect of the proportion of legumes in the sward on animal output. In *Changes in sward composition and productivity* (Occasional Symposium no. 10, British Grassland Society) (ed. A. H. Charles & R. J. Haggar), pp. 101–109. Hurley: British Grassland Society.
Wheeler, J. L. 1958 The effect of sheep excreta and nitrogenous fertilizers on the botanical composition and production of a ley. *J. Br. Grassld Soc.* **13**, 196–202.
Williams, R. J. B. & Cooke, G. W. 1972 Experiments on herbage crops at Saxmundham, 1967–71. *Rothamsted Experimental Station. Report for 1971*, part 2, pp. 95–121.
Witty, J. F., Day, J. M. & Dart, P. J. 1977 The nitrogen economy of the Broadbalk experiments. *Rothamsted Experimental Station. Report for 1976*, part 2, pp. 111–118.