RHIZOBIAL NITROGEN FIXATION ON MINED LANDS[1]

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Rhizobial nitrogen fixation and its role in building up fertility on mine spoils are described using data from acetylene reduction and nitrogen accumulation studies in the UK. Major factors affecting fixation were spoil moisture levels, sunshine hours and phosphate supply. Legume/grass plots relying on rhizobial fixed nitrogen accumulated more recent organic and mineralizable nitrogen than grass only plots relying on fertilizer nitrogen. The management of grass/legume swards is discussed on the basis of these findings.

INTRODUCTION

It is almost 100 years since the nature of the legume-Rhizobium symbiosis was discovered (Hellriegel and Wilfarth 1888) and over 50 years since observations were made in the Illinois coalfield of the lush growth of and profusion of large nodules on the roots of the legumes Cassia and Melilotus spp. in worked out mine areas (McDougall 1918; Croxton 1928). Despite these early observations and the recognition that most lands after mining are severely nitrogen deficient (Whyte and Sysam 1949), very little research has been carried out on rhizobial nitrogen fixation on mined lands.

Legumes have, however, much to offer in land reclamation. Their herbage is consistently high in nitrogen and is particularly nutritive to grazers. Nitrogen fixed by the legume-Rhizobium symbiosis is released to the soil and companion vegetation by the decay of nitrogen rich roots, nodules and aerial parts of the plant and excretion of nitrogenous compounds from the nodules (Virtanen and Laine 1939; Walker et al 1954; Butler and Bathurst 1956). In soil economic benefits have been suggested through a switch from fertilizer based grass swards to grass/clover swards in some grazing systems (Doyle and Morrison 1983).

The development of the acetylene reduction technique for measuring nitrogen fixation (Hardy, Burns and Holsten 1973) has resulted in much research into the factors affecting fixation. Many of the factors found to reduce nitrogen fixation in soils such as drought, extreme temperatures, waterlogging and harsh physical and chemical conditions are common on mined lands. Studies using the acetylene reduction technique on disturbed lands are, however, few. Skeffington and Bradshaw (1980) used acetylene reduction for evaluating the effectiveness of various legume and non-legume associations on china clay waste whereas Heilman and Ekuan (1982) and Rother et al. (1983), respectively, assessed fixation by Alnus spp. on colliery spoil and by white clover on soils contaminated with cadmium, lead and zinc. Yamanaka and Holl (1984) also measured acetylene reduction activity while assessing the affects of N and seeding rate in grass/legume mixtures on colliery spoil in greenhouse experiments.

This paper, based on acetylene reduction and nitrogen accumulation studies in field trials on colliery spoil in the UK, aims to elucidate the important factors governing rhizobial nitrogen fixation on mined lands and to point to management methods to overcome them.

MATERIALS AND METHODS

Trial Site

All field measurements were carried out at a field trial established in 1975 at Thorne in South Yorkshire, UK. The trial was of a randomized block split plot design incorporating four blocks (replicates), twelve main plot treatments (six nitrogen sources in combination with two phosphate levels) and four subplot treatments (grass cultivars). Further details of the trial are given in Palmer and Iverson (1983). The research reported here was confined to Lolium perenne cv. S23 and Festuca rubra cv. S59 subplots of the ammonium sulphate treatments (receiving nitrogen fertilizer every year) and the white clover (Trifolium repens) cv. S100 treatments (which received no nitrogen fertilizer) at both phosphate levels (32.8 and 98.4 kg P ha^{-1} at establishment in 1975 only).

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[1]Paper presented at the American Society for Surface Mining and Reclamation 1985 National Meeting. [Denver, Colorado, October 8-10, 1985].

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Proceedings America Society of Mining and Reclamation, 1985 pp 283-288
DOI: 10.21000/JASMR85010283

Nitrogen Fixation

Nitrogen fixation was measured by the acetylene reduction method weekly between May and November 1980. On each assay date, spoil cores were taken from each subplot mentioned above, incubated in the field for one hour and assayed on return to the laboratory by gas chromatography. The spoil cores were analysed for moisture, pH, electrical conductivity, potential inorganic (incubated) and available nitrogen, phosphate and water soluble cations. Spoil temperature measurements were made at 0.5, 5 and 10 cm depth at each assay and daily rainfall, sunshine hours, maximum and minimum air temperature data was obtained from a nearby meteorological station. Full details of methods are given in Palmer and Iverson (1983).

Nitrogen Accumulation

Potential inorganic and available (and hence mineralizable) nitrogen levels were determined at depths up to 80 cm in 1981 and recent organic nitrogen accumulation was determined on samples collected in 1982. Methods of nitrogen analysis used are presented in Palmer et al. (1985).

RESULTS

Nitrogen Fixation

Fixation rates fluctuated considerably over the monitoring period but were greater on white clover plots than on ammonium sulphate plots and on high phosphate plots than on low phosphate plots at almost every assay (fig. 1). Nitrogen fixation rates on ammonium sulphate plots were never significantly different from zero. Over 80% of the nitrogen fixation occurring in the top 20 cm of spoil ocurred in the top 7.5 cm, the sampling depth for the acetylene reduction assay (fig. 2).

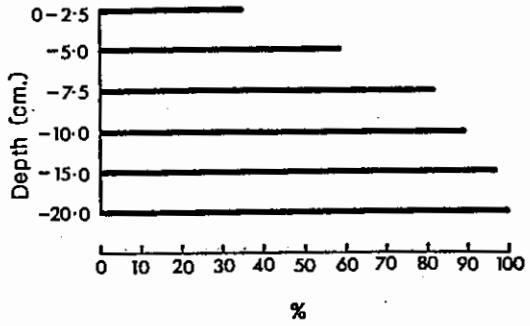

Figure 2.--Cumulated % with depth of the total nitrogen fixation occurring in the top 20cm of spoil at Thorne in 1980.

Correlation and regression analysis revealed that although climatic variables were the dominant factors affecting nitrogen fixation, the relationships were very different depending on whether data from the whole monitoring period or just the midsummer period were taken into account (fig. 3). This was because the relationship for the whole monitoring period was highly influenced by low fixation rates associated with cloudy, wet weather in autumn at the end of the monitoring period. Ignoring this period allowed the factors causing low fixation rates associated with dry weather in midsummer to be more fully assessed (fig. 3). The major factors were in fact rainfall and spoil moisture which were obviously associated. A lag in the effect of rainfall on fixation was apparent in the multiple linear regression relationship for the period between harvests (fig. 3). A similar effect was found with spoil moisture. Fixation was not significantly correlated with moisture on the assay day ($r = -0.003$) but was with spoil moisture two weeks previously ($r = 0.625$, $p < 0.05$). Spoil phosphate level and pH were also represented in the multiple linear regression equation. Troughs in nitrogen fixation were observed after both harvests (fig. 1).

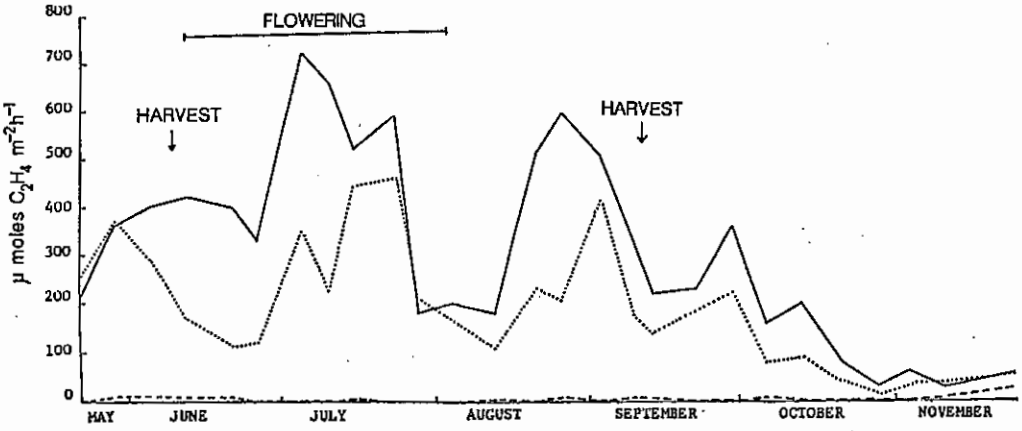

Figure 1.--Acetylene reduction levels during 1980 on white clover plots at high (——) and low (·····) phosphate and on ammonium sulphate plots (- - -) on colliery spoil at Thorne.

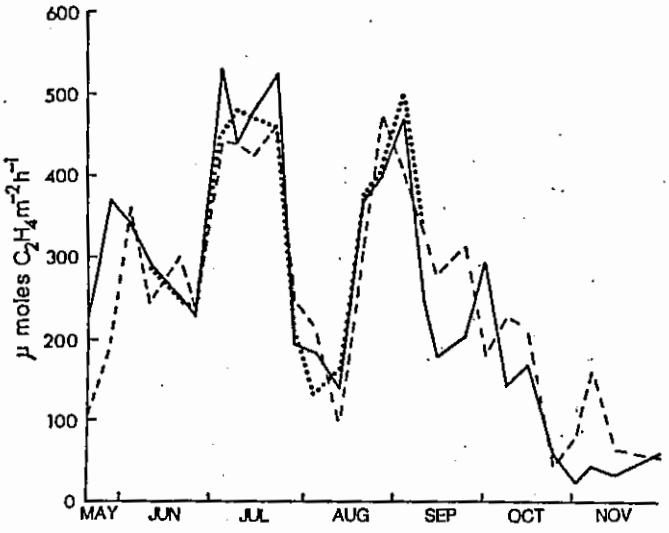

Figure 3.--Mean acetylene reduction on white clover plots at Thorne in 1980 (——) compared with relationships fitted using stepwise multiple linear regression. (– – –) fitted line for the whole monitoring period; (·····) for period between harvests only.

Fitted model	% of variance accounted for
Whole sampling period	
y = + 0.722 SH(2)	33.7
+ 0.308 R(24)	44.3
- 2.198 M	61.8
+ 1.727 PM	70.6
+17.093	
Period between harvests	
y = + 0.542 CR(27)	71.3
+ 1.090 SHO	79.7
+18.283 pH	85.8
-98.475	

SH(2) - Cumulated sunshine hours at assay and 2 days previous, R(24) - Cumulated rainfall 24 days prior to assay (mm), M - Spoil moisture (%), PM - Spoil phosphate (ug P g^{-1}), CR(27) - Cumulated rainfall 12-27 days prior to assay (mm), SHO - Sunshine hours on assay day, pH - Spoil pH.

Nitrogen Accumulation

Spoil nitrogen determinations on the samples used for nitrogen fixation revealed that significant mineralization of nitrogen only took place on white clover plots. Those plots to which fertilizer had been applied were almost totally dependent on fertilizer nitrogen supply, very little nitrogen being mineralized from organic matter throughout the monitoring period (fig. 4). Mineralizable nitrogen determinations down the spoil profile showed that most mineralization was taking place at the surface on both white clover and fertilizer plots. Mineralization on both was however greater than in unameliorated spoil (fig. 5). The profiles indicated that mineralization on white clover plots and in unameliorated spoil might be taking place down to about 35 cm but the amounts were small and within the bounds of experimental error at this site.

Determination of the accumulation of recent organic nitrogen indicated that nitrogen had accumulated since initiation of the trial seven years earlier in white clover and ammonium sulphate plots and in unameliorated spoil (fig. 6). Accumulation on white clover plots was, however, almost 50% greater than that of fertilizer plots despite the white clover plots never having received fertilizer nitrogen and the ammonium sulphate plots having received 500 kg N ha^{-1} over the same period. Accumulation in unameliorated spoil represented a rate of less than 10 kg N ha^{-1} $year^{-1}$.

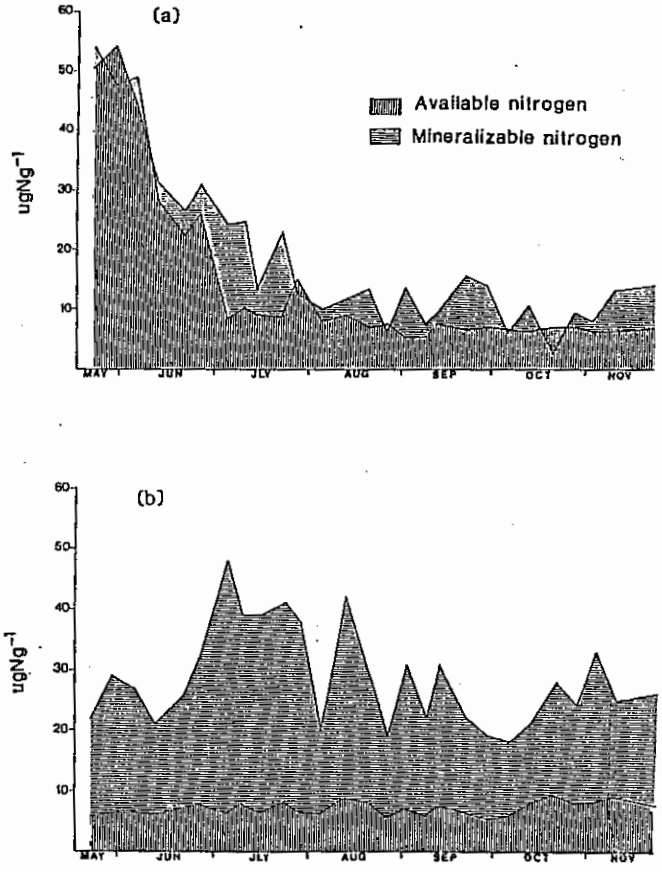

Figure 4.--Available and mineralizable nitrogen levels on (a) ammonium sulphate plots and (b) white clover plots on colliery spoil at Thorne in 1980. Fertilizer was applied to ammonium sulphate plots in mid-May 1980; no fertilizer had ever been applied to white clover plots.

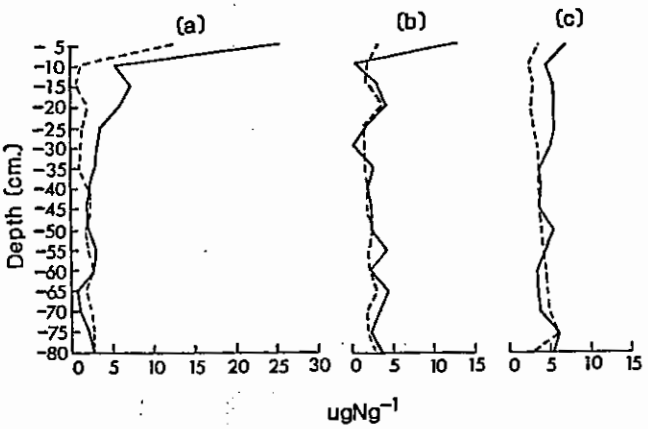

Figure 5.--Available (----) and potential inorganic (——) nitrogen levels down the spoil profile on white clover (a) and ammonium sulphate (b) plots and in unameliorated spoil (c) at Thorne in 1981. Mineralizable nitrogen is the difference between available and potential inorganic nitrogen.

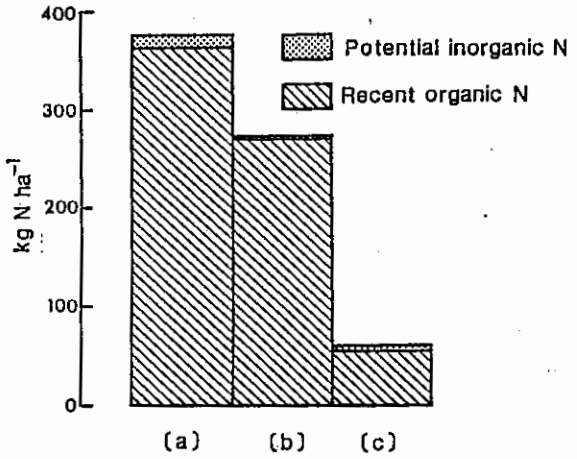

Figure 6.--The levels of recent organic nitrogen, indicating the proportion which is potentially mineralizable, accumulated to a depth of 10cm on white clover (a) and ammonium sulphate plots (b) and in unameliorated spoil (c)in the seven years since trial establishment at Thorne.

DISCUSSION

The Management of Legumes on Mined Lands

The data presented here from nitrogen fixation and spoil nitrogen accumulation studies in swards relying on legumes or fertilizer for their nitrogen source allow management options for maximizing nitrogen fixation in legume-based swards to be discussed.

Nitrogen Fixation

It is clear that climatic factors, particularly the amount of sunshine and rainfall and associated spoil moisture, are important factors governing fixation. The reclamation practitioner can do nothing about the amount of sunshine at a site but may have some control over spoil moisture levels. The data presented show that there was a lag period of about two weeks in the effect of rainfall/spoil moisture levels on fixation. Similar responses have been found in controlled environment experiments (Engin and Sprent 1973) and emphasize the fundamental nature of the effect of moisture levels on fixation. Cultural practices which conserve spoil moisture should therefore be encouraged wherever possible.

Two non-climatic factors appeared in the multiple linear regression equations over which the manager has some control. These were phosphate level and pH. It is evident from the effect of imposed phosphate treatments that phosphate level is an extremely important factor in governing fixation. Even five years after the application of phosphate at establishment the differential between the two levels was reflected in nitrogen fixation levels. The appearance of phosphate in the multiple linear regression equation relates, however, to changes in phosphate concentration throughout the monitoring period rather than to superimposed phosphate levels. This suggests that small changes in phosphate concentration were having an effect on fixation. Interestingly, phosphate concentration over the monitoring period was also correlated with moisture variables. It is possible that changes in spoil moisture were having an effect on short term phosphate concentration and this in turn was affecting nitrogen fixation.

The role of pH is less easy to explain, the range only being between 5.52-6.11. This is, however, around the range at which losses of productivity of legumes have been noticed in poorly buffered soils (Haynes 1983). pH analysis was performed on bulk samples so the range for individual samples would have probably been greater and itself reflect an even greater range at microsite level. Maintaining an adequate phosphate level and pH should therefore be primary concerns during the management of legume based swards.

Troughs in nitrogen fixation were noted here after both harvests. Similar troughs after defoliation have been noted by other workers (Moustafa et al. 1969; Halliday and Pate 1976) although some have recorded no effect (Haystead and Marriot 1978). Troughs are likely to be greatest where defoliation is infrequent. Generally with pasture legumes like white clover, frequent cutting or grazing maintains a high proportion of legume in the sward and will thus benefit the overall level of fixation.

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Nitrogen Accumulation

The spoil nitrogen accumulation studies show that clover plots have a number of advantages over those receiving fertilizer nitrogen. In particular they have a consistently higher level of mineralizable nitrogen over the growing season. Potential inorganic (mineralizable + available) nitrogen levels oven the season were positively correlated with nitrogen fixation ($r = 0.420$, $p<0.05$). Comparison of figures 1 and 3 show that mineralizable nitrogen was highest when nitrogen fixation was at its peak. Release of mineral nitrogen from decaying roots and nodules would also be expected to occur when fixation was low and this is reflected here by substantial mineralization in the autumn. Nitrogen fixation activity down the spoil profile also agrees well with mineralizable nitrogen levels down the profile. Both only occur to any extent in the top 10cm of spoil.

The nitrogen accumulation studies indicata that nearly 400 kg ha^{-1} of recent organic nitrogen have accumulated on white clover plots in the seven years since establishment. This is very low compared to the 2000 kg ha^{-1} or so of organic nitrogen found in most soil associations (Stevenson 1982). Nevertheless, the level of accumulation on white clover plots which had never received fertilizer nitrogen is considerably greater than that of fertilizer plots having received 500 kg N ha^{-1} since establishment. Both white clover plots and fertilizer plots had accumulated considerably more recent organic nitrogen than unameliorated spoil. The low level of mineralizable nitrogen on fertilizer plots would suggest, however, that the nitrogen accumulated on these plots is largely immobilized, probably in material of a high carbon to nitrogen ratio. Evidence of this occurring in fertilizer based swards on mined lands has been reported by Weider et al. (1983).

The data presented here suggests, therefore, that rhizobial nitrogen fixation on mined lands can fulfil the role outlined in the Introduction of accumulating nitrogen and providing a consistent nitrogen supply without the need for regular fertilizer nitrogen inputs. These studies also suggest, however, that despite these benefits, nitrogen accumulation in legume-based swards seven years old is still considerably less than is usual for soils. In these swards, therefore, nitrogen supply and sward performance will be more sensitive to management practices which reduce nitrogen fixation than similar swards on fertile soils. Management practices must therefore be such that they use every available opportunity to maximize fixation.

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