

Harnessing Biological Nitrogen Fixation in African Agriculture



Challenges and Opportunities



edited by
Sheunesu M. Mpeperekwi
and
Fred T. Makonese

Harnessing Biological Nitrogen Fixation in African Agriculture

Challenges and Opportunities

Sixth International Conference of the African Association
for Biological Nitrogen Fixation
12–17 September, 1994, Harare, Zimbabwe
Selected Papers

Edited by
Sheunesu M. Mpeperekwi
and
Fred T. Makonese



UNIVERSITY OF
ZIMBABWE
Publications



All rights reserved. No part of this book may be reproduced in any form or by electronic or mechanical means, including information storage and retrieval systems, without permission in writing from the publisher, except by a reviewer, who may quote brief passages in a review.

© S. M. Mpeperekki and F. T. Makonese, 1998

ISBN 0-908307-58-6

First published in 1998 by
University of Zimbabwe Publications
P.O. Box MP 203
Mount Pleasant
Harare
Zimbabwe

Typeset by University of Zimbabwe Publications
Printed in Zimbabwe by Mazongororo Paper Converters (Pvt.) Ltd

1.3 Feedback inhibition of nitrogen fixation by plant and environmental factors

S. M. Liya, A. A. Agboola and C. T. I. Odu
Department of Agronomy, University of Ibadan, Nigeria

Summary

A literature review provides evidence that legumes have greater requirements for water and nutrients, and are more sensitive to toxicities of Mn and Al than non-legumes. Furthermore, legumes are more exigent when fixing N_2 , than when using nitrogen in the soil solution, although certain species and varieties of nitrogen-fixing legumes are less sensitive to stress than others. When a large proportion of the nitrogen assimilated is derived from symbiotic nitrogen fixation, the legume's ability to procure water and nutrients may be reduced for three reasons; poor root growth, a changed cation-anion balance within the plant and acidification of the rhizosphere. It is therefore postulated that, in the field, nitrogen fixation is subject to a series of feedback-inhibition mechanisms, probably controlled chiefly by the hormone abscissic acid (ABA). Nitrogen fixation causes the plant to grow faster, and increases the need for nutrients and water. When any one becomes deficient, ABA is produced, which promotes nodule senescence at concentrations which stimulate fine root growth. If the fine roots find another source of the limiting factor, nitrogen fixation will resume. Such cyclic processes have been documented for water stress. They may also occur due to nutrient deficiencies and to rhizosphere acidification in acid soils. One consequence of feedback inhibition is that legumes can only derive a high proportion of their nitrogen from fixation when conditions for growth are good. When growth conditions are poor, for instance in drought-prone kaolinitic soils low in organic matter, nodulation will be rare. Legume varieties should be selected and bred (in combination with suitable root nodule bacteria) for greater symbiotic activity under such conditions. Cultural practices can also reduce stress, e.g. the addition of crop residues and other plant material to acid soils.

Introduction

The notion that resource-poor farmers in developing countries can use nitrogen-fixing legumes to produce their own green manure fertiliser and nutritive pulse and forage crops on marginal soils is very seductive. Dobreiner and Campelo (1977) were enthusiastic. They pointed out that a large variety of nitrogen-fixing grain, forage and forest legumes is available in the tropics. If these were exploited to produce protein for man and livestock and to increase soil fertility, agricultural development in the tropics would take off. The legumes would supply free nitrogen and all that would be needed, would be attention to mineral nutrition, management practices and providing the right rhizobia.

Research into nitrogen fixation has attracted much interest and a lot of research funding. Despite the vast amount of research that has been carried out, it is depressing that tropical agriculture has not benefitted (Dommergues and Ganry, 1986). With few exceptions, the roots of grain legumes growing on tropical small-holdings are rarely well-nodulated (Giller and Wilson, 1991) while alley cropping trials on farmers fields using nitrogen-fixing trees mostly give negative results (Ong, 1994).

Most research into ways to increase nitrogen fixation has assumed that the main reason why legumes do not fix more nitrogen is the low symbiotic efficiency of naturally occurring root nodule bacteria and their failure to continue to fix nitrogen when soil mineral nitrogen is available (Elkan, 1992). Too few attempts have been made to understand how nitrogen fixation is limited by other aspects of host physiology and environment in order to recommend improved management practices and better breeding programmes (Dommergues and Ganry, 1986). The purpose of this review is to highlight the paradoxical relationship between symbiotic nitrogen fixation and environmental stress; with the onset of nodule activity, legumes grow faster, but this increases their requirements for water and nutrients. At the same time, the onset of symbiotic activity tends to reduce water and nutrient availability by reducing root growth, increasing the dangers of Mn and Al toxicity by rhizosphere acidification which may slow down cation uptake by increasing the cation/anion ratio in plant tissue. The growth potential of nitrogen-fixing plants may therefore be limited by nitrogen fixation itself. A better understanding of how, when and why nodule function is regulated would enable nitrogen fixation to make a greater contribution to tropical agriculture. The notion that it may not be in the interest of the host plant to form nodules under certain conditions of soil and climate has not received sufficient attention.

Environmental factors limiting nitrogen fixation

The most important factors limiting nitrogen fixation in Africa are moisture stress, low soil P availability, high soil mineral nitrogen and soil acidity (Dommergues and Ganry, 1986; Parker, 1985). According to Parker (1985) legumes have the same nutrient requirements as other plants and in about the same quantities, with the exceptions of molybdenum and cobalt.

Against this, there is considerable evidence that the onset of nitrogen fixation increases a legume's requirements of both nutrients and water. Symbiotically dependent plants may require more P.

In soyabeans dependent on symbiotically supplied N, the P requirement is increased by 50% (Cassman *et al.*, 1981). Most tropical legumes require available soil P levels of 20–25 ppm (Russel, 1978) compared to 10–16 ppm, the critical value for soil P for cereals in southern Nigeria (Adeoye and Agboola, 1985). Legume species and varieties, however, differ in their tolerance of P stress (Munns and Franco, 1982). As P fertiliser is expensive, N inputs obtained from symbiotic fixation should not therefore be regarded as free (Larue and Patterson, 1981). If plant growth rate is increased by nitrogen fixation, the K requirement increases (Duke and Collins, 1985; Liya *et al.* 1990). Adequate K improves the ability of the plant to withstand water stress, the critical level of soil K for legumes is higher than that for grasses and cereals (Mengel and Kirby, 1987), and legumes recover K more slowly (Lambert and Linck, 1964). In the Nigerian savanna, it has been found that the critical soil K level for *Andropogon* grass was 0.18 cmol/kg while that of the legume *Stylosanthes* was 0.20 cmol/kg. Unless soil K is adequate legumes do not persist in pastures (Mengel and Kirby, 1987). Application of P and K fertiliser (70–80 kg each per ha) to an acid Ultisol in Brazil, doubled the amount of nitrogen fixed by a variety of tropical forage legumes and increased the percentage of plant nitrogen derived from the symbiosis (Cadisch *et al.*, 1986). Legumes are also more sensitive to soil deficiencies of S, Mo, Zn and Cu (Russel, 1978).

There is evidence that nitrogen fixation increases the water requirements of legumes. When plants are dependent on symbiotically supplied N, their transpiration rate increases (Insam, 1986). In general, legumes are characterised by a profligate use of water (Sprent, 1976). Their water use efficiency is lower than that of other species. Ludlow and Wilson (1972) showed that the tropical grasses *Sorghum album* and *Pennisetum purpureum* have transpiration ratios (9 g of water transpired per g of CO₂ fixed) of just under 80, compared to 180 for the tropical legumes *Calpogonium mucunoides* and *Glycine wightii*. The amount of water required to produce one kg of dry matter was 700 l for a sward of sole *Stylosanthes* sp compared to 305–340 l for a sward of *Pennisetum* sp. In addition to its higher water requirements, nitrogen fixation by legumes is more sensitive to moisture stress than nitrogen uptake (Sprent, 1976).

Legumes also tend to be particularly sensitive to Mn and Al toxicity. Nodulation and nitrogen fixation are more sensitive to Mn toxicity than host plant growth but legume species differ in their susceptibility which is genetically determined (Kenehiro *et al.*, 1978). When the Al concentration in the rooting medium is high, dry matter production by tropical pasture legumes, dependent upon symbiotically fixed N, decreases more than that of legumes dependent on combined N (de Carvalho, 1981; Ogata *et al.*, 1986). In Burundi, Wouters *et al.*, (1986) observed that an aluminum-tolerant variety of *Phaseolus vulgaris* introduced by CIAT still produced 90% of maximum yield at 73% aluminum saturation of the exchange complex, however, nodulation was completely suppressed once the percentage aluminum saturation reached 33%. It appears, therefore, that legume crops are less adapted to dry and infertile soils when they are fixing nitrogen than when taking nitrogen from the soil.

Hormonal changes in nitrogen-fixing plants subject to stress

The ammonia produced in the root nodules is potentially toxic to the host plant. The supply of organic ions needed for its detoxification may not be adequate when growth slows down and it becomes desirable for nitrogen fixation to stop. Nitrogen-fixing plants are believed to control their nodule activity by the hormone abscissic acid (ABA). ABA may inhibit nodule development (Sutton, 1983), cause nodule dormancy (Mascarenhas and Pollen, 1985) and nodule senescence (Sutton, 1981). However, it stimulates fine root growth (Gaither *et al.*, 1975) at concentrations which inhibit nodule development (Mansfield, 1987).

Abscissic acid is believed to be a carotene degradation product (Hess, 1975) and is high in stressed plants when leaves wilt or yellow (Most, 1971; Mansfield, 1987). It follows that when nitrogen-fixing plants experience water or nutrient stress, ABA will be produced, nodules will cease to function but fine root growth may be stimulated. The effects of ABA are counteracted by cytokinins.

Relationships between nodule activity and fine root growth

Nodulation and fine root growth are controlled by the same genes (Nutman, 1948), and are often positively related. However, numerous studies have reported reduced root growth by symbiotically dependent legumes (Nutman, 1948; Dart and Pate, 1959). Reasons for this discrepancy were found by Cassman *et al.*, (1980) in soyabeans subjected to different degrees of P and water stress, there was an inverse relationship between nodule biomass and fine root growth. It was concluded that well-nodulated soyabeans growing in infertile soils, subject to water shortage, would suffer more from drought and nutrient deficiency than nitrate dependent plants.

This conclusion is supported by the findings that supernodulating soyabean mutants, are characterised by the ability to nodulate very profusely, even in the presence of nitrate (Carroll *et al.*, 1985). These mutants have lost the usual hormonal control of nodulation. Gresshoff *et al.*, (1985) attributed their poor field performance to reduction in root growth.

It appears therefore, that though root growth may not be affected by nodulation when water and minerals are abundant, when plants are stressed ABA will be produced and result in root growth taking precedence over nodulation. In tropical agro-ecosystems nutrient and/or water stress is frequent. There is therefore likely to be a general tendency for plants to reduce nodule activity in favour of enhanced root growth.

This explains the frequently made observation that in natural eco-systems, very little nitrogen is fixed unless P and water stresses are removed, as shown with Acacia trees in the Australian outback (Hansen and Pate, 1987), nitrogen-fixing trees in the African savanna (Hogberg, 1987), and beans in farmers fields in Rwanda, Tanzania and Kenya (Ssali, 1988; Giller and Wilson, 1991).

Rhizosphere acidification by nitrogen-fixing plants

Most nitrogen-fixing plants acidify their rhizospheres. Nitrate generally serves as the main anion balancing cation uptake, so in plants dependent on nitrogen fixation, cation uptake exceeds anion

uptake. To balance the equilibrium in the plant, protons are expelled into the rhizosphere (Israel and Jackson, 1978), and the rhizosphere soil acidifies. In a pot experiment carried out by Mengel and Steffens (1982), the pH of soil supporting symbiotically dependent legumes dropped from 7.2 to 4.5 in 14 months, no pH drop occurred in soil fertilised with $(\text{NH}_4)_2\text{NO}_3$. In Nigeria, Juo (1980) reported that after 4 years under monocropped soyabeans, pH dropped from an initial value of 6.5 to 5.9. Under monocropped maize pH only fell to 6.1. Under pigeon pea, pH dropped to 5.6. Greenhouse experiments have shown that soyabean and alfalfa can lower pH by one unit in 70 days (Sprent, 1979).

Schaller (1987) pointed out that the degree of rhizosphere acidification is proportional to the buffering capacity of the soil, i.e. its cation exchange capacity, which is determined in kaolinitic tropical soils primarily by organic matter content. In all soil types rhizosphere acidification was shown by Schaller to be greatest when initial soil pH is around 6, because of more variable charge at this pH (Schaller, 1987).

Acidification of the rhizosphere soil may affect plant growth by affecting the profile of availability of the major soil nutrients. In neutral and calcareous soils, plants may grow better because of increased P availability. In acid soils, on the other hand, rhizosphere acidification will tend to reduce plant growth by provoking Al and/or Mn toxicity and Ca and/or Mg deficiency (Sanchez, 1976). The negative effects of toxicities and deficiencies generally become apparent when pH falls below about 5.5 (Sanchez, 1976; Mengel and Kirby, 1987). Symptoms of Al toxicity (yellowing leaves and reduced root growth) generally appear at a pH of around 5.4 but can occur up to pH 6.0 if Al is abundant. The severity of the symptoms is correlated with the ratio $\text{Al}_{3+}/\text{CEC}$ rather than the absolute value of Al_{3+} (Koal and Kassam, 1978). Cowpeas and pigeon peas are more tolerant of high soil Al than soya or field beans (Sanchez, 1976). Cowpeas and groundnuts are more tolerant than pigeon peas and Mung beans (Chong *et al.*, 1988).

Manganese toxicity interferes with auxin production by the shoot tip and restricts root growth. It can occur at soil pH of between 5.5 and 6.0 if soil Mn is sufficiently abundant (Sanchez, 1976), or if the soil is periodically waterlogged (Willett, 1983; Giller and Wilson, 1992). On sedimentary soils in southwestern Nigeria, Adeoye and Agboola (1985) reported increasing Mn toxicity to maize as soil pH fell below 6.0.

For legumes, the availability of Mo, which is essential for nitrogen fixation, decreases when the pH falls below 5.5 (Mengel and Kirby, 1987). Molybdenum deficiencies have been reported on groundnuts in Senegal and Nigeria, particularly in soils high in iron oxides which fix molybdenum (Kowala and Kassam, 1978).

It appears, therefore, that when soil pH falls below 5.0–5.5, nitrogen fixation will slow down. This, however, is not invariable. The optimum pH for some nitrogen-fixing crops such as lupins can be reported that *Albizia lebbeck* and other nitrogen-fixing trees nodulated in soil of pH 4.9, even when inoculated with acid sensitive rhizobium strains. Apparently the trees were capable of raising the pH of their rhizospheres. The genetic resources and physiological processes which enable such legumes to continue to fix nitrogen in acid soils require detailed study.

There is evidence that more acidity is generated by temperate than tropical legume species, and even among the latter, species and varieties differ in the amount of acidity generated (Israel and Jackson, 1978).

Changed cation-anion balance of nitrogen-fixing plants

As described above, symbiotic nitrogen fixation tends to cause cation uptake to exceed anion uptake. In such cases, the mechanism used by plants to maintain the pH of their tissue near to neutrality is to synthesise organic anions, such as malate to neutralise the excess of cations. Malate synthesis is accompanied by CO_2 assimilation (Smith and Raven, 1979). Organic acids accumulate in red clover plants dependent on symbiosis (Hauter and Steffans 1985). As malate synthesis requires energy, its production cannot continue indefinitely. There is reason to believe that when

NO_3 is not taken up, the drawing force for K uptake is slowed down (Israel and Jackson, 1979). This partly explains the high K requirements of nitrogen-fixing plants described above.

In a study of early growth of leguminous trees in the field, Liya *et al.*, (1990) observed that during the rainy season the growth of the nitrogen-fixing species was limited by soil K, while growth of the non-nitrogen-fixing species was independent of soil K. Subsequent growth of the nitrogen-fixing species during the dry season, when all nodules shrivelled up, bore no relationship with soil potassium (Liya, unpublished results).

Feedback inhibition of nitrogen-fixation

To summarise the findings reviewed above, the onset of nitrogen fixation increases the need for water, phosphorus, potassium, and other nutrients and renders the plant more vulnerable to toxicities of Mn and Al. Meanwhile, as a result of symbiotic activity, the plant's ability to procure extra water and nutrients tends to be reduced by three separate processes: reduced root growth, acidification of the rhizosphere, and changed cation-anion balance with the plant (Fig. 1.3.1). In addition, when plants are dependent on root nodules for their nitrogen supply, soil nitrate, instead of being a growth factor, may become a toxic substance which can reduce the growth of nodulated plants (Herridge and Brockwell 1988).

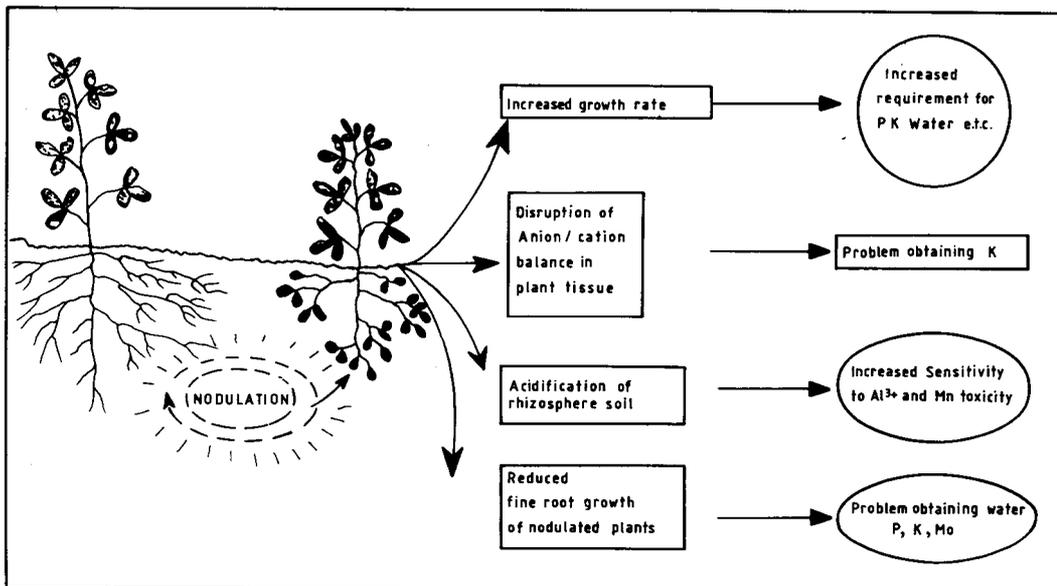


Fig. 1.3.1: Consequences of nodulation

It follows that nitrogen fixation is a self-limiting process; nodule activity appears to be controlled by a series of feedback inhibition mechanisms. Nitrogen fixation accelerates growth and increases the need for nutrients and water. When any one of these becomes deficient abscisic acid is produced, symbiotic activity ceases, but fine root growth may be stimulated. Nodule activity may resume if the enlarged root system gives access to new sources of the limiting factor (Fig. 1.3.2).

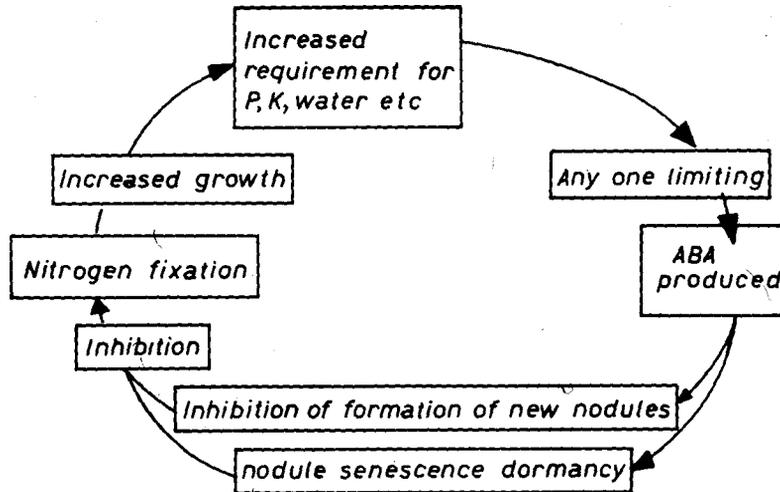


Fig. 1.3.2: Feedback inhibition of nitrogen fixation by increased requirement for limiting factors

Feedback inhibition can be caused by soil moisture deficiency. Nitrogen-fixing plants use soil moisture more rapidly than those which are not fixing nitrogen. Unless rainfall or irrigation is regular, soil moisture will then be depleted and nitrogen fixation will then come to a stop. Leaves will yellow from lack of N, abscisic acid will be produced, stimulating fine root growth. If these roots find moist soil, the plant may renodulate. Such a sequence has been reported with subterranean clover growing in a drying soil in Australia. Davey *et al.* (1989) observed that nodules in the topsoil shrivelled as the soil dried, but new roots grew into the moist subsoil and new nodules formed on these roots. The same phenomenon was observed in leguminous trees growing in a seasonally dry climate in Nigeria (Ball, 1985; Liya, 1994). Response of nodulated legumes to P and K deficiencies by similar cyclic processes may explain the perplexing problem of poor nodulation of *Phaseolus* beans in the field, even in the presence of numerous compatible and effective rhizobia, unless P is applied (Ssali, 1988; Giller and Wilson, 1992).

Rhizosphere acidification is likely to cause feedback inhibition of nodule activity in any soil with a low cation exchange capacity and a pH below 6.0 if nitrogen fixation causes the rhizosphere pH to drop below 5.5. In such cases Mn and/or Al toxicities are probable, unless the CEC is increased by the addition of organic material. Deficiencies of Ca, Mg and Mo are also likely, which may slow down or prevent nitrogen fixation without affecting nitrate uptake. When nitrate is taken up, however, rhizosphere pH may increase (Jarvis and Robson, 1983), making it possible for nodule activity to restart (Fig. 1.3.3). During nitrate uptake, grasses and cereals raise soil pH more than legumes (Jarvis and Robson, 1983). Hence intercropping legumes with C3 plants may help to prevent feedback inhibition of nitrogen fixation by soil acidity.

Another well-documented phenomenon is what might be termed the feedback de-inhibition of nodule activity operated by soil mineral nitrogen. Numerous previous investigations have shown that high plant available nitrogen in soil inhibits nodule development, nitrogenase activity and accelerates nodule senescence in the short term. Over longer periods, however, high available N leads to the formation of more nodules and greater nitrogen fixation, an effect due to enhanced plant growth, which depletes soil mineral nitrogen prior to nodulation, and produces a larger plant (Dart, 1977; Munns, 1977; Agboola, 1978; Jones, 1985; Bibson, 1987) (Fig. 1.3.4).

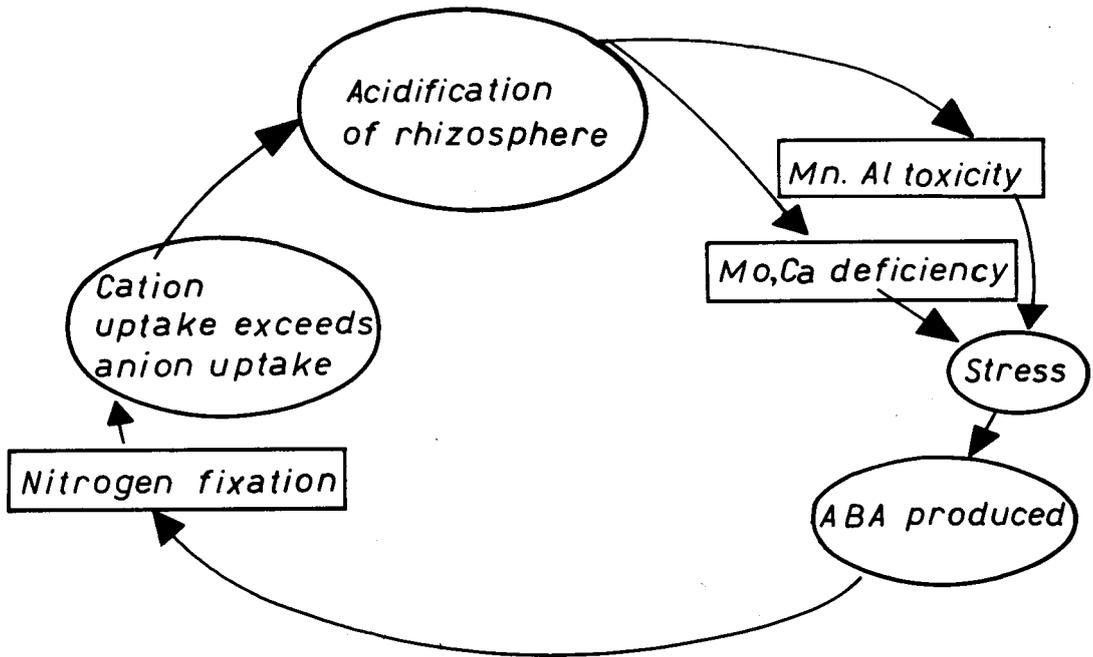


Fig. 1.3.3: Feedback inhibition of nitrogen fixation by nutrient stress caused by rhizosphere acidification

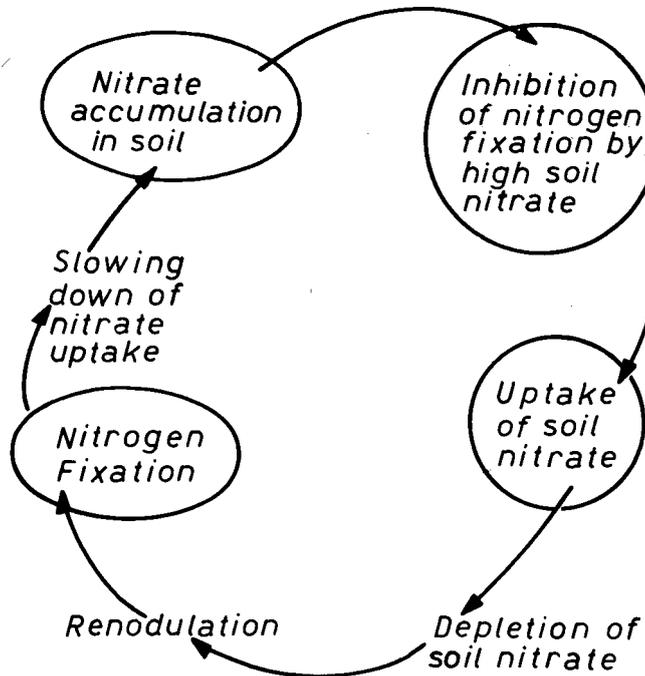


Fig. 1.3.4: Feedback inhibition of nitrogen fixation by high soil nitrate

Methods of reducing feedback inhibition by plant husbandry

Given the sensitivity of legumes to nutrient imbalances caused by soil acidity, which are aggravated by symbiotic activity itself, it is not surprising to find that in acid tropical soils, soil organic matter has a significant effect in stimulating nodulation and nitrogen fixation, by increasing the CEC and the buffering capacity of the soil and masking variably charged sites on iron and other amorphous sesquioxides (Sanchez, 1976). In acid soils in Australia, nodulation is often restricted to the top 4 cm where organic matter is the highest (Richardson *et al.*, 1988). Addition of organic materials can also play an important role. For example, the application of 4 t/ha of millet straw to groundnut in a sandy acid soil in Niger enhanced nodulation and nitrogenase activity. Nitrogen accumulation by the crop was doubled due to decreased availability of Al and Mn coupled with increased availability of Mo (Rebafk *et al.*, 1993). Wheat straw is as good as liming for preventing symptoms of Al toxicity in soyabean growing in an acid ultisol (Ahmad and Tan (1986). Different results may be obtained in Alfisols, where Al toxicity is not a problem. Here, nodule biomass and acetylene reduction per plant may be increased or decreased by mulches depending on their chemical composition and the legume species and varieties used as test plants. Mulches of rice straw and sawdust, which immobilised mineral nitrogen in the soil, generally increased nodule activity per plant by 2–4 times (IITA, 1976).

Moisture stress is another major factor limiting nitrogen fixation in the tropics. Short droughts are common even in humid regions. Moisture stress should be avoided by mulching, shading and proper K fertilisation.

Conclusion

1. Legumes dependent on symbiotic nitrogen fixation are more sensitive to limiting factors such as moisture stress, nutrient deficiencies and toxic effects than plants dependent on soil nitrogen.
2. Therefore, little nitrogen can be fixed in dry infertile soils with low CEC, prone to drought, because nitrogen fixation aggravates the other environmental limitations and therefore may cause feedback inhibition.
3. As a consequence of feedback inhibition, both the amount of nitrogen which can be fixed by a particular legume-rhizobium symbiosis and the proportion of plant nitrogen derived from the symbiosis will depend on the particular soil in which the experiment is carried out and water availability throughout the experiment. This should be taken into consideration when testing the nitrogen-fixing capacity of legume varieties, and rhizobial strains.
4. Nitrogen-fixing legume varieties should be selected and bred:
 - (a) which do not acidify their rhizospheres;
 - (b) whose root growth is not restricted by nodulation when subject to P and water stress, and
 - (c) whose capacity for cation uptake is not restricted by nitrogen fixation.
5. Unless such plants can be found, feedback inhibition in problem soils might be avoided in breeding programmes if we are to concentrate on legumes which used soil nitrogen synergistically with nitrogen fixation, i.e. which rely on symbiotic nitrogen fixation for < 50% of their requirements.
6. As the sensitivity to stress of a nitrogen-fixing legume is influenced by the hormone balance of the plant, the possibility of reducing sensitivity to stress by inoculation with cytokinin producing strains of micro-organisms (rhizobia, other bacteria and mycorrhiza) should receive attention.

Because of the sensitivity of legumes fixing nitrogen to limiting factors, the tacit assumption that symbiotic nitrogen fixation can increase the agricultural production of small farmers working infertile, marginal tropical soils requires re-examination.

References

- ADEOYE, G.O. AND A.A. AGBOOLA. 1984. Relationship between relative yield and soil pH, available P, K, Zn and Mn and maize earleaf content of P, Cu and Mn on sedimentary soils in SW Nigeria. *Fert. Res.* 5, 109–113.
- ADEOYE, G.O. AND A.A. AGBOOLA. 1985. Critical levels for soil pH, available P, K, Zn and Mn and maize earleaf content of P, Cu and Mn on sedimentary soils in SW Nigeria. *Fert. Res.* 6, 65–71.
- AGBOOLA, A.A. 1978. The effect of soil organic matter content on cowpea response to nitrogen application. *Agron. J.* 70, 25–78.
- AHMAD, F. AND K.K. TAN. 1986. Effect of lime and organic matter on soyabean seedlings grown in aluminum toxic soil. *Soil Sci. Soc. Am. J.* 50, 656–661.
- BALL, B. 1965. Root distribution and nutrient cycling of some tree-shrubs suitable for alley cropping in the humid tropics. M. Sc. thesis, University of Guelph, Canada.
- CARROLL, B.J., D.L. MCNEIL AND P.M. GRESSHOFF. 1985. A supernodulating and nitrate tolerant symbiotic (nts) soyabean mutant. *Plant Physiol.* 78, 34–40.
- CASSMAN, K.G., A.S. WHITNEY AND K.P. STOCKINGER. 1980. Root growth and dry matter distribution of soyabean as affected by phosphorus stress, nodulation and nitrogen source. *Crop Sci.* 20, 239–240.
- CASSMAN, F.G., A.S. WHITNEY AND R.L. FOX. 1991. Phosphorus requirements of cowpea and soyabean as affected by mode of N nutrition. *Agron. J.* 73, 17–22.
- CERVILHO, DE M.M., C.J. EDWARDS, C.S. ANDREW AND C.J. ASHER. 1991. Aluminum toxicity, nodulation, and growth of *Stylosanthes* species. *Agron. J.* 73, 261–265.
- DART, P. 1977. Infection and development of leguminous nodules. In R.W.F. Hardy and W.S. Silver (eds). *A treatise on nitrogen fixation. 3. Biology*. John Wiley and Sons, New York, pp 367–472.
- DAVEY, A.G., A.P. HENDERSON AND R.J. SIMPSON. 1989. Nodulation and growth of subterranean clover (*Trifolium subterraneum* L.) in a drying soil. *Soil Biol. Biochem.* 21, 1–8.
- DOBEREINER, J. AND A.B. CAMPELO. 1977. Importance of legumes and their contribution to tropical agriculture. In R.W.F. Hardy and A.H. Gibson (eds). *A treatise on Dinitrogen Fixation. 4. Agronomy and Ecology*, John Wiley and Sons, New York. pp 191–220.
- DOMMERMUES, Y.R. AND F. GANRY. 1986. Biological nitrogen fixation and soil fertility maintenance. In A.O. Mokwunye and P.L.G. Vlek (eds.). *Management of N and P fertilisers in sub-Saharan Africa*. Martinus Nijhoff, Holland. pp 95–115.
- DUKE, S.H. AND M. COLLIN. 1985. Role of potassium in legume dinitrogen fixation. In: *Potassium in agriculture*, ASA, Madison USA. pp 443–465.
- ELKAN, G.H. 1992. Biological nitrogen fixation in tropical eco-system: An overview. In K. Mulongoy, G. Gueye and D.S.C. Spencer (eds). *Biological nitrogen fixation and the sustainability of tropical agriculture*. Wiley and Sons Chichester, UK. pp 27–40.
- FOY, C.D., R.L. CHANEY AND M.C. WHITE. 1978. The physiology of metal toxicity in plants. *Ann. Rev. plant Physiol.* 29, 511–566.
- GAITHER, D.H., D.H. LUTZ AND L.E. FLORENCE. 1975. Abscissic acid stimulates elongation of excised pea root tips. *Plant Physiol.* 55, 948–949.
- GIBSON, A.H. 1987. Evaluation of nitrogen fixation by legumes. In G.H. Elkan (ed.) *Symbiotic nitrogen fixation technology*. Marcel Dekker, Inc. New York. pp 321–369.
- GILLER, K.E. AND K.J. WILSON. 1991. *Nitrogen fixation in tropical cropping systems*. C.A.B. International, Wallingford, UK.
- GRESSHOFF, P.M., D.A. DAY, A.C. DELVES, A.P. MATHES, J.E. OLSSON, G.D. PRICE, K.A. SCHULLER AND B.J. CARROLL. 1985. Plant host genetics of nodulation and symbiotic nitrogen fixation in pea and soyabean. In H.J. Evans, P.J. Bottomley and W.E. Newton (eds.). *Nitrogen fixation research progress*. Martinus Nijhoff, the Hague pp 19–25.
- HANSEN, A.P. AND J.S. PATE. 1987. Comparative growth and symbiotic performance of seedlings of *Acacia* species in defined pot culture or as natural understorey components of a eucalyptus forest eco-system in S.W. Australia. *J. Exp. Bot.* 38, 13–25.
- HAUTER, R. AND D. STEFFENS. 1985. (In German) Influence of mineral and symbiotic nitrogen nutrition on proton release of roots, phosphorus uptake and root development of red clover. *Z. Pflanzernaehr. Bodenk.* 148, 633–646.

- HERRIDGE, D.F. AND J. BROCKWELL. 1988. Contributions of fixed nitrogen and soil nitrate to the nitrogen economy of irrigated soyabean. *Soil Bio. Biochem.* 20, 711–717.
- HESS, D. 1975. *Plant Physiology*. Springer Verlag, Berlin.
- HOGBERG, P. 1986. Nitrogen fixation and nutrient relations in savanna woodland trees in Tanzania. *J. of Applied Ecology*. 23, 675–688.
- IITA. 1976. Effects of mulches on legume nodule functioning. *Annual Report for 1975*, pp 24–25.
- IMSANDE, J. 1968. Rapid nitrogen fixation during pod fill enhances the transpiration rate and net photosynthetic output of soyabean. In: H. Bothe, F.J. de Bruijn and E.W. Newton (eds.). *Nitrogen fixation: 100 years after*. Gustava Fisher, Stuttgart and New York. p. 817.
- ISRAEL, D.W. AND W.A. JACKSON. 1978. The influence of nitrogen nutrition on ion uptake and translocation by leguminous plants. In: C.S. Andrew and E.J. Kamprath (eds.) *Mineral nutrition of legumes in tropical and subtropical soils*. CSIRO, Australia. pp 113–128.
- JARVIS, C.S. AND A.D. ROBSON. 1983. The effect of nitrogen nutrition on the development of acidity in western Australian soil. *Austr. J. Agric. Res.* 34, 341–365.
- JONES, R.M. 1985. Effect of nitrogen and inoculum levels on establishment and nodulation of leucaena. *Leucaena Research Reports* 6, 8–10.
- JUO, A.S.R. 1980. Nitrogen profile in a kaolinitic alfisol under fallow and continuous cultivation. In T. Rosswall (ed.) *Nitrogen cycling in West African eco-systems*. SCOPE. Royal Swedish Academy of Science. pp 181–189.
- KANEHIRO, Y., J.L. WALKER AND M. ASGHAR. 1984. Special considerations in utilising tropical legumes in pastures. 2 Edaphic factors. In: B.L. Burt, P.P. Ratan, L. Walker and M.W. Silvey (eds.). *The role of Centrosema, Desmodium and Stylosanthes in improving tropical pastures*. Bowker Publishing Company, Epping, England. pp. 197–219.
- KOWAL, J.M. AND A.H. KASSAM. 1978. *Agricultural ecology of savanna — a study of West Africa*. Clarendon Press, Oxford.
- LAMBERT, R.G. AND A.J. LINCK. 1964. Comparison of uptake of P₃₂ and K₄₂ by alfalfa and oat roots. *Plant Physiol.* 39, 920–924.
- LARUE, T.A. AND T.G. PATTERSON. 1981. How much nitrogen do legumes fix? *Adv. Agron* 34, 15–38.
- LIYA, S.M., K. MULONGOY, C.T.I. ODU AND A.A. AGBOOLA. 1990. The influence of some parameters of soil fertility on early growth of *Leucaena leucocephala* and *Cassia siamea*. In: M. Gueye, K. Mulongoy and Y. Dommergues (eds.) *Maximiser la fixation biologique d'azote pour la production agricole et forestiere en Afrique*, AABNF 1–12 November, 1988, Dakar, Senegal. *Collection Actes de l'USRA* 2, 250–256.
- LUDLOW, M.M. AND G.L. WILSON. 1971. Photosynthesis of tropical pasture plant. *Austr. J. Biol. Sci.* 24, 1077–1087.
- MANFIELD, T.A. 1987. Hormones as regulators of water balance. In: F.J. Davies (ed.). *Plant hormones and their role in plant growth and development*. Martinus Nijhoff. Publishers. Dordrecht, Holland. pp 411–430.
- MASCARENHAS, J.P. AND C.D. POLLEN. 1985. Symbionts and symbiont-induced structures. In: R.P. Harris and D.M. Reid (eds.) *Hormonal regulation of development III. Encyclopedia of Plant Physiology New Series Vol. 11*. Springer Verlag, Berlin. pp 579–588.
- MENGEL, K. AND E.A. KIRBY. 1987. *Principles of plant nutrition*, International Potash Institute, Bern.
- MENGEL, K. AND D. STEFFANS. 1982. (In German). Relationship between cation/anion uptake and the release of protons by roots of red clover. *Z. Pflanzernaehr. Bodenk* 145, 22–236.
- MOST, B.H. 1971. Abscissic acid in mature apical tissue of sugar cane and in leaves of plants subjected to drought. *Planta (Berl.)* 101, 67–75.
- MUNNS, D.N. 1977. Mineral nutrition and the legume symbiosis. In: R.W.F. Hardy and A.H. Gibson (eds.). *A treatise on dinitrogen fixation Section IV. Agronomy and Ecology*. John Wiley and Sons, New York. pp 353–391.
- MUNNS, D.N. AND A. FRANCO. 1982. Soil constraints to nitrogen fixation. In P.H. Graham and S.C. Harris (eds.) *Biological Nitrogen Fixation for tropical Agriculture*, CIAT, Colombia. pp 133–152.
- NUTMAN, P.S. 1948. Physiological studies on nodule formation. I. The relation between nodulation and lateral root formation in red clover. *Ann. Bot. Lond.* 13, 261–283.

- ONG, C. 1994. Alley cropping — ecological pie in the sky? *Agroforestry Today* 6, 8–10.
- PARKER, C.A. 1985. Nitrogen fixation for developing countries with special reference to Africa. In : H.O. Ssali and S. Keya (eds.) *Biological nitrogen fixation in Africa*. The Nairobi MIRCEN, Kenya. pp 10–29.
- REBAFKA, F-P., B.J. NDUNGURU AND H. HARSCHNER. 1993. Crop residue application increases nitrogen fixation and dry matter production in groundnut (*Archis hypogaea*) grown on an acid sandy soil in Niger, West Africa. *Plant and Soil* 150, 213–222.
- RICHARDSON, A.E., A.P. HENDERSON, G.S. JAMES AND R.J. SIMPSON. 1988. Consequences of soil acidity and the effect of lime on the nodulation of *Trifolium subterranean* L. growing in an acid soil. *Soil Biol. Biochem.* 20, 439–445.
- RUSSEL, J.S. 1976. Soil factors affecting the growth of legumes on low fertility soils in the tropics and subtropics. In: C.S. Andre and E.J. Kamprath (eds.) *Mineral nutrition of legumes in tropical and subtropical soils*. CSIRO, Australia. pp 75–92.
- SANCHEZ, P.A. 1976. *Properties and management of soils in the Tropics*. John Wiley New York.
- SCHALLER, G. 1987. pH changes in the rhizosphere in relation to the pH-buffering of soils. *Plant and Soil* 97, 439–444.
- SMITH, F.A. AND RAVEN, J.A. 1979. Intercellular pH and its regulation. *Ann. Rev. Plant Physiol.* 30, 289–311.
- SPRENT, JANET, I. 1976. Water deficits and nitrogen-fixing root nodules. In: T.T. Kozłowski (ed.) *Water deficits and plant growth*. Volume IV Academic Press. New York. pp 291–315.
- SPRENT, JANET, I. 1986. Nitrogen fixation in a sustainable agriculture. *Biol. Ag. and Hort.* 3, 153–165.
- SSALI, H. 1988. *Rhizobium phaseoli* inoculation trials on farmer's fields in Kenya. *E. Afr. Agric. For. J.* 53, 151–157.
- SUTTON, W.D. 1983. Nodule development and senescence. In : W.J. Broughton (ed.) *Nitrogen Fixation*. Volume 3. Legumes. Clarendon Press, Oxford. pp 144–212.
- WOUTERS, J.F.R., M. WAKANA AND L. OPDECAMP. 1986. Sensibilite due haricot *Phaseolus vulgaris* a la concentration en aluminium des sols de la region des grands lacs. *Tropicultura* 4, 20–26.
- WHELAN, A.M. AND M. ALEXANDER. 1986. Effects of pH and high Al, Mn and Fe levels on the survival of *Rhizobium trifolii* and the nodulation of subterranean clover.
- WILLETT, I.R. 1983. Oxidation-reduction reactions. In : *Soils an Australian Newpount*. CSIRO, Melbourne. pp 417–426.



This work is licensed under a
Creative Commons
Attribution – NonCommercial - NoDerivs 3.0 License.

To view a copy of the license please see:
<http://creativecommons.org/licenses/by-nc-nd/3.0/>

This is a download from the BLDS Digital Library on OpenDocs
<http://opendocs.ids.ac.uk/opendocs/>