Non-symbiotic Nitrogen Fixation and Soil Fertility

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Abstract

Soils in the semi-arid tropics (SAT) are low in nitrogen and yet they continue to support most of the world's millets and sorghum production with virtually no addition of fertiliser. Legumes seem to play only a small role in the maintenance of soil fertility under the present management and cropping patterns. High levels of N can accumulate in soil under certain fallows planted to grasses such as *Andropogon gayanus*. Mineralisation rates of 4 to 5% per annum provide up to 70 kg N/ha per annum as nitrate, some of which may leach to soil depths of 1 to 2 m. Nitrogen removal in pearl millet or sorghum crops in such soils can be as much as 150 kg N/ha, depending on the cultivar and the season. Since inputs from other sources such as rainfall are small, the maintenance of equilibrium levels of soil N depends on biological N_2 fixation. Bacteria associated with the root systems of sorghum, millets and some tropical grasses are involved, as well as blue-green algal crusts.

Long-term N balances for crop production, although difficult to measure, are necessary for estimating the amounts of N fixed. In pot culture at ICRISAT, positive balances have been obtained in vermiculite for sorghum of 108 mg N/plant, for pearl millet of 22 mg N/plant and a cross between pearl millet and Napier grass (Napier bajra) of 361 mg N/plant. In an irrigated long-term trial with several tropical grasses in an Alfisol, with no N fertiliser added, Napier bajra has produced 136 tonnes/ha in 30 months, containing 1185 kg N/ha. Blue-green algal crusts are estimated to contribute about 28 kg N/ha per annum to this total.

The acetylene reduction assay for nitrogenase activity has shown that the roots of many SAT plants stimulate N_2 fixation. The assay is very sensitive, but there are many precautions to be observed when it is used in field studies. Nitrogenase activity varies throughout the day, as well as over the season, making it difficult to estimate an integrated value for N fixation over time. The use of ^{15}N is a much more promising approach.

There is variability between lines of millet and sorghum in stimulating N_2 fixation; about 7% of the 618 different lines tested showed high nitrogenase activity. At ICRISAT we are examining whether this can be exploited in a selection and breeding programme for increased N_2 fixation. Activity is favoured in moist, warm (*ca.* 35° C) soil and decreased by high levels of mineral N.

Many different kinds of bacteria closely associated with the roots of SAT plants show nitrogenase activity. This makes choice of an inoculum strain difficult. Responses to inoculation in terms of increased dry-matter production and N uptake have been obtained in pot experiments with sorghum, millet and Napier bajra grown in Alfisol soil and vermiculite.

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The semi-arid tropics (SAT) produce 95% of the world's millets and 60% of the world's sorghum, from a total cropped area of about 70 million ha. Both cereals respond to inputs of N, yet almost all the production is without the use of fertiliser, relying on N mineralised from organic matter in the soil. Crop production is thus dependent on the content of N in the soil, the rate at which organic matter is mineralised, and the soil N replenished. There is a dearth of information on the inputs and losses of N under different SAT cropping systems. A key issue is whether the existing systems are in a state of balance, or whether, even with the present low productivity levels, yields are gradually declining because crops are mining the soil supplies of N and other nutrients. Biological N_2 fixation is the major input, and we try to assess the evidence that non-symbiotic N_2 fixation associated with sorghum, millets and some grasses, is contributing to the long-term maintenance of soil N levels. The possibility of a significant input from non-symbiotic N_2 fixation has only recently gained support and is based mainly on attempts to construct soil N balances for SAT production systems, and the finding that many tropical plants with the C_4 photosynthetic pathway, including pearl millet and sorghum, stimulate nitrogenase activity as measured by acetylene reduction around their roots.

In this paper we briefly review the inputs and losses of N from SAT soils, the evidence for non-symbiotic N_2 fixation from N balances and the acetylene reduction assay, the distribution of N_2-fixing bacteria in soil and the responses to inoculation with N_2-fixing bacteria.

Nitrogen Turnover

Soil N Levels

Because the amounts of nitrogen involved are small there is much uncertainty about the dynamics of N in SAT soils.

The total amount and the dynamics of N in the soil profile are influenced by both climate and soil texture through their effects on the vegetation. In the semi-arid tropics (SAT)[1] soils with the largest total amounts of N are in regions where rainfall exceeds 1000 mm per annum. The major soil orders in the SAT are Alfisols, Aridisols, Entisols, Oxisols and Vertisols; all of these generally have low organic matter contents (Kampen and Burford 1980; Swindale 1982). For 295 sites in SAT West Africa, the mean N content of the topsoil was 0.051%, with values ranging down to 0.008% N (Jones 1973). In India, the medium Vertisols contain 0.02 to 0.044% N in the top 30 cm and Alfisols 0.03 to 0.04% N (e.g. Jenny and Raychaudhuri 1960; Venkata Rao and Badiger 1977; see also Indian Soil Science Research Review for 12th Cong. Int. Soc. Soil Sci. 1982).

Information on the rates of mineralisation of the organic matter in SAT soils

1. Under Troll's classification the SAT are defined as areas where monthly rainfall exceeds potential evapotranspiration for 2 to 7 months and the mean monthly temperature is above 18°C. The areas with 2 to $4\frac{1}{2}$ wet months are called dry semi-arid tropics and those with $4\frac{1}{2}$ to 7 wet months are called wet-dry semi-arid tropics.

and of the movement of nitrate and ammonia in the profile is restricted to very few sites (Ghana—Nye and Greenland 1960; Australia—Wetselaar 1961, 1967; Senegal—Blondel 1971a, 1971b, 1971c, 1971d; Nigeria—Jones 1975).

In many SAT soils there is a flush of mineralisation of organic matter in the surface soil layers at the start of the rainy season (e.g. Wetselaar and Arndt 1960). In sandy soils, mineralisation (Blondel 1971d) and nitrification (Blondel 1971e) were apparently stimulated by plants and addition of N fertiliser (Blondel 1971c). Annual mineralisation rates are about 4 to 5% (Wetselaar 1967; Jones and Wild 1975). The farmer's problem is to sow early enough so that crops can use the nitrate released before it is leached out of the root zone, in a situation where uncertain rainfall and consequent uncertain soil moisture availability increase the riskiness of planting early in the rainy season. Generally, soil N content decreases during cultivation of cereals, particularly following clearing of a forest, and increases under bush fallow (Sahasrabuddhe and Kanitkar 1933; Nye and Greenland 1960; Charreau and Fauck 1970; P. Sibaud personal communication; Bartholomew 1977), but in some situations millet and sorghum have been cropped continuously for many years with little or no fertiliser addition and little change in yields. For a soil containing 0.03% N, a net mineralisation rate of organic N of 4% per annum in the top 30 cm soil yields about 50 kg N/ha.

Losses of N

Leaching and removal of N in crop material are the major avenues for loss of N from SAT soils. Small amounts are lost through burning and denitrification. Little is known about the magnitude of losses from leaching but they are believed to be small under crops which take up the nitrate. The increase of clay content with soil depth reduces the rate of leaching in deeper soil layers (see Jones and Wild 1975) and is one of the major factors determining differences between soils in rates of nitrate movement. Studies with lysimeters filled with three sandy soils in Senegal showed that from 5 to 30 kg N/ha leached annually to a depth of 2 m (C. Charreau, personal communication). Whether this is then unavailable to plants is a moot point, since roots of long-duration sorghum, pearl millet and some grasses can grow to this depth. Blondel (1971a, 1971b, 1971c, 1971d) showed that there was considerable change in nitrate and ammonia levels at different soil depths during the season in Senegal in three different soil types.

Wetselaar (1961) at Katherine, in semi-arid northern Australia, and Simpson (1963) in Uganda, found that nitrate moved towards the soil surface by capillary rise during the dry season, so that although there may be rapid leaching of the nitrate built up by mineralisation early in the season, this may not become totally unavailable to plants. The quantities of N involved in this upward movement of nitrate and the interaction with soil type are not known.

Although few measurements seem to be available, denitrification is believed to be small (Greenland 1962), except that when large amounts of N fertiliser were added to a sandy ferruginous Dior soil in Senegal, denitrification amounted to 45% of the total N loss (Ganry et al. 1978). Denitrification may also be

considerable in deep Vertisols, where anaerobic pockets develop during periods of waterlogging.

The burning that occurs periodically in the bush fallow results in N losses estimated at 12 to 20 kg N/ha for each burn (J. Krul, personal communication; Vidal and Fauche 1961; Norman and Wetselaar 1960).

Removal of N in crops

In most SAT production of millet and sorghum, most of the stover as well as the grain is removed at harvest. The amounts of N removed may be large and in some experiments much more N was removed than one would estimate as being available to the crop as nitrate derived from mineralisation. Even if more N is mineralised than is removed by the crop in a particular season, without a net addition of N to balance this pattern of removal over time, soil N levels and crop production would gradually decline.

The uptake of N into above-ground plant parts of sorghum and millet was studied in two experiments at ICRISAT Center near Hyderabad. Five African selections of pearl millet grown in an Alfisol containing about 50 kg N/ha as nitrate measured in the top 1 m of soil with a further 20 kg N/ha added as fertiliser, produced a mean yield of 1070 kg grain/ha and 4460 kg total above-ground biomass, containing 45 kg N/ha. Thus about 64% of the potentially available N was used by the crop. There were significant differences between the varieties in N uptake, with a range from 37 to 58 kg N/ha (G. Alagarswamy and F.R. Bidinger, personal communication). If we assume the same efficiency of uptake of the 50 kg/ha of mineral N in the soil with no fertiliser added, this soil should support pearl millet crops producing about 680 kg/ha grain and a biomass of 2870 kg, a value of the same order as that for overall annual production in India (440 kg/ha) and West Africa (570 kg/ha), averaged over the years 1974 to 1978.

A tall, late-maturing, local Indian sorghum cultivar grown at ICRISAT Center in a low-fertility Alfisol with 20 kg N/ha added as fertiliser yielded only 300 kg/ha grain, but produced 15 tonnes dry matter/ha, containing 134 kg N. The top 30 cm soil contained about 0.03% total N, and the field had been under cereal cultivation in the previous year with only 20 kg N/ha fertiliser added. For 13 different genotypes in the same experiment, the dry-matter production varied from 5.4 to 15 tonnes/ha, with N uptake from 66 to 155 kg/ha (N. Seetharaman and F.R. Bidinger, personal communication).

Soil N Gains

The major input is biological N_2 fixation, with a very small amount coming through rainfall and dust. Rainfall away from the sea appears to contribute little N—1 to 4.5 kg N/ha per annum (Wetselaar and Hutton 1963; Jones and Bromfield 1970)—although much of this may be derived from the soil in the surrounding area and hence is not a net addition to the region as a whole. Fertiliser use is virtually nil, although rainfed high-yielding varieties of sorghum and millet in India may receive 20 to 40 kg N/ha.

Nitrogen is fixed biologically by symbiotic, nodulated legumes and non-legumes such as *Casuarina* spp; by the non-symbiotic systems such as blue-green algal crusts on the soil surface; and by the bacteria living freely in the soil and closely associated with the roots of grasses, cereals and some dicotyledonous plants--the associative N_2-fixing systems.

The input from grain legumes is limited, as they are sown over a much smaller area than the cereals and the scope for rotation of cereals with legumes to improve soil N is limited. In India, cereals and grain legumes are often intercropped, while in Africa, such intercrops often rotate with unplanted (bush) fallow. In the intercropped situation it is doubtful if much N is transferred from the legume to the cereal during the growing season. The amount of N fixed and returned to the soil depends very much on the type of legume and its grain yield. For example the traditional, spreading, late-maturing cowpeas often intercropped in Africa would return more N than improved early types with a high harvest index. No measurements of the N fluxes in such systems have been published.

Very few experimenters have examined the residual benefit for subsequent crops of the N fixed by grain legumes. At Samaru in northern Nigeria, Jones (1974) showed that where no N fertiliser was applied on an Alfisol, maize following groundnut yielded 1 tonne/ha more, and took up 30 kg/ha more N than maize following sorghum or cowpea. In India on an Alfisol, an increase in yield of 45%, or 650 kg/ha, grain was obtained for millet following groundnut over millet following maize (ICRISAT 1977). On a sandy loam soil in north India, yields of millet after groundnut were 23% more; after cowpea, 24% more; after pigeonpea, 12% more than yield of millet after millet. For the cowpea and groundnut, this was estimated equivalent to the application to the millet of 60 kg N/ha as fertiliser (Giri and De 1979). On a Vertisol at Hyderabad in peninsular India, Kumar Rao and Dart (1980) found that pigeonpea had a residual effect on a maize crop equivalent to the effect of 40 kg N/ha supplied as fertiliser to the maize.

Under sown legume forage crops which are grazed by animals, the amounts of N accumulated are much greater and were as much as 106 kg N/ha in the soil under *Stylosanthes humilis* in northern Australia (Wetselaar and Norman 1960; Wetselaar et al. 1973).

Bush fallows usually contain few legumes (Jones and Wild 1975; J. Krul personal communication). Surprisingly, the amounts of N that may build up in the topsoil under grass during a bush fallow (uncropped) period may be as large as the residual effect of the grain legumes; e.g., 59 to 83 kg N/ha per annum under *Andropogon gayanus* at Samaru is northern Nigeria (Jones 1971; Jones and Wild 1975). Similar accumulation of N under planted stands of *A. gayanus, Pennisetum purpureum* and *Panicum maximum* were obtained at three sites in Ghana (Nye 1958). However, it is not clear how much of this accumulation is a net gain from biological N_2 fixation and how much is from the redistribution of N from lower depths in the profile by the process of uptake by grass roots and redeposition as litter.

Evidence for Non-Symbiotic Fixation from N Balances

Moore (1963) found a positive N balance over a 4-month period for the soil-plant system in pot experiments with finger millet, *Eleusine coracana,* that extrapolated to a gain of 112 to 148 kg N/ha. Unfortunately, there are virtually no published N-balance studies for dryland cereal crop production under tropical field conditions. Such experiments are difficult to conduct, as they need to run for more than one season and require a rigorous schedule for sampling the soil (see Vallis 1973) if they are to reliably measure soil N changes of 20 to 50 kg N/ha per annum.

In several field experiments with sorghum, addition of small amounts of N fertiliser have resulted in increase in the N content of the crop greater than the amount of added N (an apparent fertiliser recovery rate of more than 100%). For example, at Nioro in Senegal in 1969, addition of 40 kg N/ha as urea to sorghum resulted in an increase in yield from 1670 to 3870 kg/ha, with an increase in N uptake by the fertilised crop of 62 kg N/ha over that of the unfertilised crop. Without the use of ^{15}N-labelled fertiliser, it is difficult to determine whether this increase is due to a priming effect of the fertiliser N on the rate of organic matter mineralisation or due to development of a root system more efficient in N uptake or due to N_2 fixation (C. Charreau, personal communication).

Working over two rainy seasons in Senegal with micro-lysimeters (34 cm diam) containing sandy, ferruginous Dior soil and left unplanted, Ganry et al. (1978) showed that there was a net gain over and above the N added of up to 46 ppm N when millet residues were added to the soil at the rate of 15 to 30 tonnes/ha on a surface area basis. This represented a net gain of 2 g N/60 kg soil. These levels of residue addition are larger than would be normally used by farmers, but the experiment illustrates that quite high levels of non-symbiotic N_2 fixation can be associated with the return of plant residues to the soil, stimulating N_2 fixation by supply of carbohydrate. Addition of plant residues such as sorghum and millet stover with a high C:N ratio also favours immobilisation of the soil nitrate, hence reducing leaching losses (Ganry et al. 1978). Appropriate soil management should result in gradual release of this immobilised N through mineralisation during the season, hopefully matching better the N demand of the crop than occurs in unamended soils.

In the old long-term permanent manurial experiments at Coimbatore in south India, there was a gain of soil N in both the control (no fertiliser) plots and the plots fertilised with N and P. After taking into account N removed in crop produce, a positive balance of N was found over and above that added in fertiliser over the years 1916 to 1959. During this period 44 crops were grown in a rotation that only included the non-legumes sorghum, *Setaria italica, Eleusine coracana, Panicum maximum,* cotton and sugarcane (Krishnamoorthy and Ravikumar 1973). In the new permanent manurial experiments the soil N content of the control plots receiving no fertiliser increased from 0.055% in 1926 to 0.058% in 1958 after 53 crops (which included sorghum, millet, *Setaria italica, Panicum maximum, Eleusine coracana* and cotton). Again for the treatments receiving 25 kg N/ha per crop as ammonium sulphate fertiliser plus 13.5 kg N/ha as cattle

manure, there was an annual gain of N in the soil of 14.9 kg/ha. The amount of N added as fertiliser balanced very closely the amount of N gained in the soil and removed in crop produce (Mariakulandai and Thyagarajan 1958).

At ICRISAT we have grown sorghum, millet and Napier bajra (*Pennisetum purpureum* x *P. americanum*) in pots in low-fertility Alfisol soil, or unsterilised, washed vermiculite and obtained substantial positive balances for N, even when the equivalent of 20 kg N/ha (53 mg per pot) was added as fertiliser. In one experiment with sorghum grown in vermiculite for 49 days, the positive N balance across all inoculation treatments was 269 mg N/pot of five plants (Table 1) when no N was applied, and 124 mg N/plant when 53 mg N/pot was applied. A substantial portion of this N was gained by the root medium (33% of the total N balance for zero N treatment), although this may result from fine roots missed from the root sample.

Table 1. Nitrogen balance for sorghum cv. CSH-5 grown in pots in unsterilised vermiculite with different inoculations and nitrogen levels.

Inoculum	N applied (mg/pot)	Total dry matter/ pot[a] (g)	N in dry matter (mg)	Net N increase/ pot[b] (mg)
Isolate from *Chloris gayana*	0	11	86	88
	53	23	161	155
Isolate from *Sorghum, halapense*	0	26	187	335
	53	31	173	100
Isolate from *Pennisetum clandestinum*	0	16	130	234
	53	17	108	204
Enterobacter spp.	0	21	133	147
	53	19	110	60
Napier bajra root extract[c]	0	39	368	542
	53	21	140	103
Boiled composite[d]	0	20	152	259
	53	13	89	243
Planted uninoculated control	0	13	99	162
	53	19	127	136
Unplanted	0			45
	53			—7.9
S.E.M. (0.05)		6.9	70	110

a. Average of four pots with five plants in each; plant age at harvest 49 days.
b. Derived from total N per pot in plant dry matter + rooting medium at harvest minus N in seed inoculum, fertiliser and rooting medium at sowing. 53 mg N/pot applied as ammonium nitrate is equivalent to 20 kg N/ha.
c. Roots of Napier bajra, a hybrid between *Pennisetum purpureum* × *P. americanum* were ground in sterile tap water, left to stand for 5 h, filtered through muslin and the extract used as an inoculum.
d. Similar populations of inocula 1-5 were mixed just prior to autoclaving for 15 min.

Table 2 shows that millet grown in vermiculite in pots also attained a positive N balance—without added N fertiliser—of 109 mg N/pot of four plants. Even with N fertiliser added at the rate of 20 kg N/ha, there was a positive N balance of

96 mg N/pot, which was not statistically different from that with no added N fertiliser.

Table 2. Nitrogen balance for millet cv. BJ-104 grown in vermiculite in pot culture at two applied N levels.

N applied		Dry matter/ pot[a]	N in dry matter	Net N increase/ pot
Kg/ha equivalent	mg/pot	(g)	(mg)	(mg)[b]
0	0	18.5	121	109
20	53	21.4	132	96
Unplanted	0	—	—	45
Unplanted	53	—	—	—7.9
L.S.D. ($P<0.05$)		1.7	10	NS

a. Plant age at harvest, 64 days; average of 28 pots each having five plants.

b. Derived from total N per pot in plant dry matter plus rooting medium minus N in seed, inoculum, fertiliser, rooting medium at sowing.

Cuttings of the hybrid Napier bajra NB-21 were grown in vermiculite and Alfisol soil. The cuttings grew without added N fertiliser to about 150 cm in 72 days before being ratooned. At final harvest, the extra N accumulated in the soil amounted to 216 mg N/pot for a single plant, without added N, and 368 mg N/plant with the equivalent of 20 kg N/ha added (Table 3). The total positive balance was 539 and 657 mg N respectively. For the vermiculite rooting medium, N accumulation with zero N treatment was 167 mg N in the medium and 361 mg N positive overall balance.

Table 3. Dry-matter production and N balance for cuttings of the hybrid *Pennisetum purpureum* × *P. americanum* grown in pots in Alfisol soil and washed, unsterilised vermiculite with different N fertiliser levels.

Medium	N applied		Total dry matter[a] (g/plant)	N in dry matter (mg/plant)	N balance[b] (mg/plant)
	Kg/ha equivalent	mg/pot			
Soil	0	0	86.7	341	539
	20	53	89.1	417	657
	120	318	137.0	785	263
Soil unplanted[c]	0	0	0	0	—375
Vermiculite	0	0	55.4	215	361
	20	53	76.0	301	303
	120	318	152.0	816	302
Vermiculite unplanted[c]	0	0	0	0	176

a. Cuttings grown for 75 days then ratooned and grown on until 194 days; mean of 10 replicate pots each containing a single plant.

b. N balance calculated from total N per pot in plant dry matter, plus rooting medium minus N in cutting, inoculum, fertiliser, rooting medium at sowing.

c. Unplanted control pots were kept moist at approx. 70% water-holding capacity.

Positive N balances have also been determined for *Paspalum notatum* cuttings grown in vermiculite with as much as 110 mg N/cutting fixed in 2 months (Dobereiner and Day 1975). Purchase (1978) also found substantial amounts of N fixation associated with *Paspalum urvillei* grown in crushed granite.

It is possible that some of this N was gained by absorption of ammonia from the air, but recent measurements for other species suggest smaller rates of uptake from this source (Wetselaar and Farquhar 1980).

We started a long-term field experiment at ICRISAT in 1978 to measure the N balance in sorghum production in an Alfisol under rainfed conditions. All above-ground plant material is removed at harvest. Plant dry-matter production and N uptake and soil N changes are measured. The same eight cultivars, chosen for their variation in agronomic characteristics and level of nitrogenase activity, are grown each year on the same plots. Fertiliser N is added as urea at the rate of 0, 20 and 40 kg N/ha. Mean initial N content of the top 0 to 30 cm of the unfertilised soil was 0.030 ± 0.004%.

Yields were very variable in the first season because of shoot-fly attack. In the second and third seasons, there were significant differences between the cultivars in grain yield and N uptake, but no significant effect of N fertiliser, possibly because of variability in stand establishment. In the second season, with no N fertiliser addition, plant dry-matter production varied from 3.05 to 10.65 tonnes/ha (Table 4) and N uptake in dry matter varied from 33 to 72 kg/ha (Table 5). IS-15165 is a late-maturing, photosensitive African entry which produced virtually no grain at Hyderabad in this season. In the third season in 1980, total dry-matter yields for the unfertilised plots were again considerable, ranging from 4.33 to 7.4 tonnes/ha. Grain yield of CSH-5 was over 2 tonnes/ha. Cumulative dry-matter yields over three seasons ranged from 11.1 to 23.4 tonnes/ha. These are surprisingly high yields for soil with low fertility. In the

Table 4. Grain and dry-matter yield (tonnes/ha) for sorghum in the second season (1979) in long-term N balance trial at ICRISAT Center, near Hyderabad, India.

Cultivar	Grain yield			Total dry matter		
	N applied (kg/ha)					
	0 N	20 N	40 N	0 N	20 N	40 N
Dobbs	0.89	1.15	1.21	10.65	12.44	11.68
IS-2333	1.76	1.68	2.37	9.91	9.08	10.85
CSH-5	3.98	3.11	3.83	9.16	8.03	9.16
IS-15165				8.60	8.17	8.01
Diallel-642	2.68	2.95	3.27	7.41	8.31	8.11
CSV-5	1.45	2.21	2.18	6.63	7.58	7.78
FLR-101	2.22	2.71	3.51	6.05	6.95	7.80
IS-889	1.39	1.74	1.93	3.05	4.29	5.28

Standard error for comparing cultivar differences in grain yield means at different N levels: 0.35; standard error for comparing cultivar differences in grain yield means at a particular N level: 0.32; standard error for comparing cultivar differences in total dry matter yields at different N levels: 1.05; standard error for comparing cultivar differences in total dry-matter yields at a particular N level: 0.91.

Table 5. Nitrogen uptake (kg N/ha) by sorghum cultivars in the second season (1979) of the long-term N balance experiment at ICRISAT Center, near Hyderabad, India.

Cultivar	N in grain			N in dry matter		
	N applied (kg/ha)					
	0 N	20 N	40 N	0 N	20 N	40 N
Dobbs	15.8	19.4	21.6	61.3	68.5	92.6
IS-2333	27.3	31.8	41.1	58.6	65.9	82.7
CSH-5	45.9	42.9	51.9	72.1	72.9	77.3
IS-15165				37.4	61.9	67.6
Diallel-642	35.0	41.6	54.0	56.7	67.4	81.0
CSV-5	20.4	36.2	37.1	56.4	78.4	90.1
FLR-101	25.4	36.1	51.5	45.5	63.6	82.8
IS-889	22.4	31.7	34.1	33.3	50.1	62.2

Standard error for comparing cultivar differences in nitrogen uptake in grain at different N levels: 5.91; standard error for comparing cultivar differences in grain nitrogen uptake at a particular N level: 5.32; standard error for comparing cultivar differences in uptake of nitrogen in the dry matter, at different N levels: 9.92; standard error for comparing cultivar differences in total nitrogen uptake in the dry matter, at a particular N level: 8.34.

absence of N_2 fixation associated with the sorghum roots, such levels of N depletion by the crop should result in a significant decrease in soil N content and a trend of decreasing yields over time.

In another long-term N-balance trial with several tropical grasses (grown in irrigated plots ranging from 5.4 to 12 m²), maximum dry-matter production has been obtained with the hybrid *Pennisetum americanum* x *P. purpureum* (JVM-2) where an equivalent of 136 tonnes/ha dry matter, containing 1185 kg N have been harvested in 30 months (Table 6). Several other grasses, including other hybrids between pearl millet and Napier grass, have also produced large amounts of dry matter in these plots, which have received a total of 120 kg P_2O_5/ha and no N fertiliser since the start of the experiment. Some entries produced much less

Table 6. Dry-matter production and nitrogen uptake by some forage grass species.[a]

Entry	Growth period (months)	Dry-matter production		N uptake	
		Cumulative (tonnes/ha)	Per day (kg/ha)	Cumulative (kg/ha)	Per day (kg/ha)
Pennisetum purpureum x *P. americanum* (cv JVM-2)	30	136.1	148	1185	1.29
P. purpureum (cv Pusa Giant Napier)	42	127.8	100	831	0.65
P. squamulatum	42	106	83	744	0.58
P. purpureum x *P. squamulatum*	42	85.2	66	667	0.52
P. massaicum	42	55.6	44	370	0.29
Cenchrus ciliaris	42	43.3	36	297	0.25
Panicum antidotale	42	27.1	21	198	0.16

a. Values derived from unreplicated plots of size 5.4 m² to 12 m² given no nitrogen fertiliser.

dry matter, for instance, *Panicum ciliaris*, with only 30 tonnes/ha, containing 175 kg N/ha.

In Rio de Janeiro State in Brazil, Kjeldahl analyses indicated that the soil under three varieties of *Digitaria decumbens* gained N at the rate of 216 to 468 g N/ha/day over 1 month, with N uptake by the forage of 1.40 to 1.50 kg N/ha per day (Schank et al. cited by Neyra and Dobereiner 1977).

This rate of N uptake is much greater than would be expected from the amount of N expected to be released by mineralisation, particularly as the soil is not disturbed by cultivation. The rates of nitrogenase activity, measured by acetylene reduction of soil cores for the plots in both India and Brazil, were high, and extrapolated to 300 to 870 g N/ha per day.

Contribution by Blue-Green Algae

Blue-green algal crusts develop on the soil surface of many cropped fields during the rainy season and after irrigation. On Alfisols their extent and N_2-fixing activity under pearl millet and sorghum was generally low, but under tropical grasses, such as *Pennisetum purpureum*, the mats may be very active, depending on the wetness of the soil surface and the extent of the plant canopy. Activity decreases rapidly as the soil surface dries out, virtually ceasing 3 days after wetting of the soil surface if the radiation levels are high (Table 7). Nitrogenase activity of these algal mats ranged from 24 to 119 mg N fixed/m^2 per day, compared with only 0.5 to 1.6 mg N/m^2 per day for surface soil without visible algal growth. In some places the algal mat covered up to 29% of the soil surface. This activity extrapolates to an upper estimate of fixation of 28 kg N/ha per year if we assume 80 days at this level of activity for an irrigated perennial grass crop, a value very similar to that estimated for algal activity under wheat on Broadbalk field at Rothamsted (Witty et al. 1977). Nitrogen fixation of this order would have little effect on the soil N balances of highly productive perennial grasses; more information is needed on the activity under cereal crops in different environments before its importance can be assessed for N balances in rainfed SAT conditions.

Table 7. Effect of soil moisture on nitrogenase activity (μmoles $C_2H_4/m^2/h$) of blue-green algae on the soil surface [a]

	Days after irrigation		
	1	2	3
Thick algal mat	817 ± 55	189 ± 38	3± 2.9
Thin algal mat	24 ± 3.7	152 ± 49	0
Soil[b]	6 ± 1.81	11 ± 6.1	0

a. Each value mean ± SEM derived from five cores each of 25.5 cm^2 area.

b. Without visible algal cover.

Measurement of N_2 Fixation

Acetylene Reduction

There are several methods for measuring N_2 fixation associated with field and pot-grown plants—chemical analyses of the soil-plant system, use of ^{15}N, and the acetylene reduction assay for nitrogenase activity. They all have a place in determining the role of non-symbiotic N fixation in crop production. Because of its sensitivity and the relative ease of its application, the acetylene reduction assay has been a widely used technique recently (*e.g.* Hardy et al. 1973). The assay involves incubation of the plant and/or soil in an atmosphere containing acetylene, which is reduced to ethylene by the nitrogenase enzyme, which also reduces N_2 to NH_3. Acetylene and ethylene are readily determined by gas chromatography (Dobereiner 1980; Knowles 1980). The acetylene reduction assay has shown that nitrogenase activity is stimulated in the root zone of many grass species, as well as millets and sorghum, which grow in the semi-arid tropics (e.g. Dart and Day 1975; Day et al. 1975; Tjepkema and Van Berkum 1977; Neyra and Dobereiner 1977; ICRISAT 1976, 1977).

There are major difficulties, however, with the use of the acetylene reduction assay in quantifying the amounts of N_2 fixed over time. The relationship between the rate of reduction of acetylene and that of N varies with the system and is seldom experimentally determined (Knowles 1981; Silvester 1981). Fewer artifacts are introduced if the system assayed is disturbed as little as possible (Balandreau 1979). Variability between plants is much less when plants are grown in pots and assayed intact, than for cores of roots plus soil of field-grown plants (ICRISAT 1979). In soil plant cores the diffusion of acetylene and ethylene into the soil may be rate-limiting (Van Berkum and Day 1980). Little is known of the pathway for diffusion of gases to and from the site of fixation and it is quite possible that as in rice, some diffusion occurs through the stem tissue (e.g. Balandreau et al. 1975; Greenwood 1968). In some cores the rate of acetylene reduction is almost linear after an initial lag of 30 to 60 minutes presumably due to the time taken for diffusion of the gases to the site of fixation, suggesting that this rate of nitrogenase activity reflects that prior to sampling (Fig. 1).

Assay of roots excised from the tops of the plant, and shaken out of the soil may lead to artifacts if long incubation times (6 h) are used, because the release of organic materials from the root may be stimulated during incubation, leading to multiplication N_2-fixing bacteria on the root surface. With longer incubations, nitrogenase activity often increases rapidly after a lag period of 6 to 12 h, although immediate acetylene reduction can be obtained for excised sorghum and millet roots (Wani et al. unpublished data).

The nitrogenase activity of excised roots is often sensitive to the partial pressure of oxygen in the atmosphere surrounding them, being greatest at a PO_2 of about 0.01 atmosphere. Many of the N_2-fixing bacteria isolated from the roots of tropical plants are facultative anaerobes or micro-aerophils; under limiting carbohydrate conditions, N_2 fixation is more efficient in terms of carbohydrate utilisation at low levels of oxygen availability (Neyra and Dobereiner 1977; Van

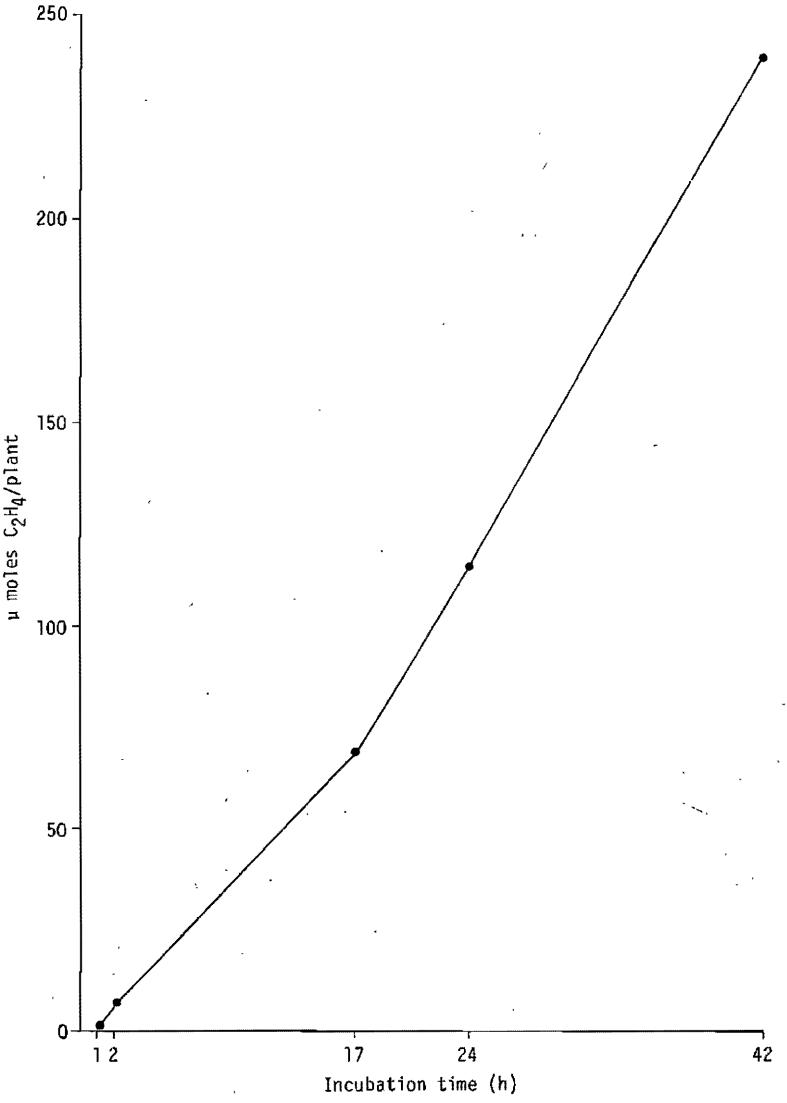

Fig. 1. Nitrogenase activity associated with a core (28 by 48 cm) of soil containing roots of *Pennisetum purpureum* Pusa Giant Napier. Above-ground parts of the plants were removed just prior to taking the core.

Berkum and Bohlool 1980). It is difficult to know what is the effective atmosphere surrounding roots in the soil; hence, it is preferable to incubate roots and soil intact in a large core which has been disturbed as little as possible.

One of the worrying characteristics associated with the acetylene reduction assay of field-grown millet and sorghum plants is the variability between plant samples and between assay times. Table 8 shows the enormous range in

nitrogenase activity associated with replicate soil-root cores of pearl millet. It is not clear whether this reflects the natural situation. However, there is also large plant-to-plant variability with *in situ* assays (Balandreau et al. 1978. Balandreau 1979) of intact plants including millet (Balandreau et al. unpublished data). It is also difficult to obtain an accurate measure of the root biomass, and it is usually only feasible to sample a small volume of the soil explored by the roots of large plants. The activity of sorghum roots after flowering extends laterally at least 30 cm beyond the single core over the crown of the plant. The single core over the crown may only represent 13 to 50% of the total activity of a millet or sorghum plant (Subba Rao and Dart 1981).

Table 8. Pearl millet lines with high nitrogenase activity.

Cultivar	Origin	N_2-ase activity[a] μg N/ core/day	Range of N_2-ase activity[b]	Active: total assays[c]	Active: total seasons[d]
P-23	Cameroon	1261	3-5031	1/1	1/1
IP-2787	Chad	559	17-1585	7/42	5/9
J-1279	India	326	6-1571	2/19	2/4
GAM-73	Senegal	246	60-370	37/290	6/9
PIB-155	India	235	198-272	6/22	4/5
DS-299	—	222	4-871	1/15	1/2
PHB-12	India	205	0.6-858	3/22	3/4
NHB-3	India	143	2-401	4/23	2/3
Souna D_2 × Ex-Bornu	—	138	108-165	4/22	2/3
70-1xJ1623-11-1(30)	ICRISAT	127	84-280	4/18	3/4
B-282	World collection from IP-282	118	85-157	6/36	3/7
J-88	India	114	0-539	5/34	4/8
J-1407	India	114	70-180	9/34	4/7
Ex-Bornu	Nigeria	105	7-252	6/43	4/9
67-B	India	104	98-112	4/30	3/6
Souna D_2x Ex-Bornu 2 (D1074-11) (5871)	ICRISAT selection	103	28-167	3/11	2/4
ICI-7543	India	102	44-208	4/14	3/4
IP-2/89	Chad	102	0-270	1/26	1/7

a. 20 x 15 cm diam cores incubated overnight (17 h) under acetylene. Values are means of 5 cores. Plants grown in Alfisol field at a population approx. 88,600 plants/ha.

b. Range for most active assay occasion.

c. No. of assays in which activity was >25μg N/core/day and total number of assays.

d. No. of seasons in which activity was >25μg N/core/day and total number of seasons assayed.

For plants grown in Alfisol soil in black plastic bags and assayed immediately after the tops were cut, nitrogenase activity was detected 16 days after planting and increased until the early grain-filling stage and thereafter declined (Fig. 2).

There is a marked diurnal periodicity of nitrogenase activity for both sorghum and millet grown in pot culture and assayed as intact plants (Fig. 3) by sealing

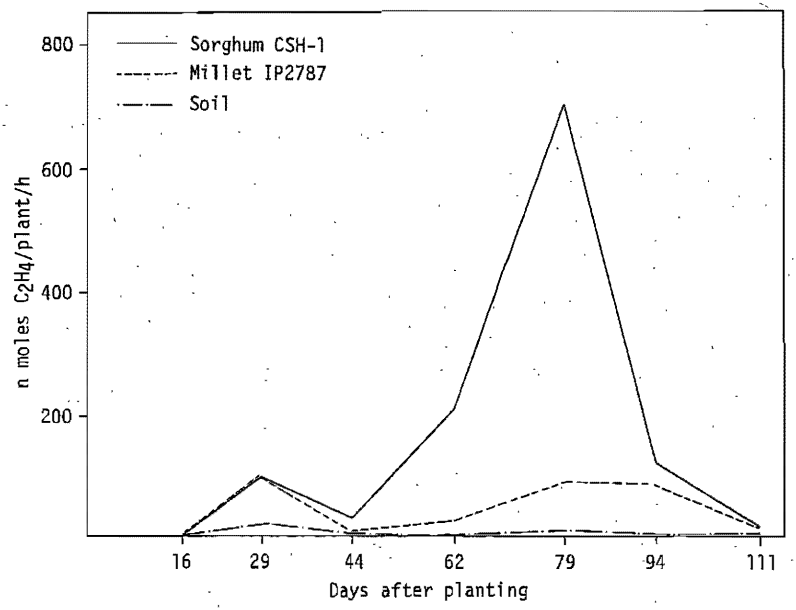

Fig. 2. Nitrogenase activity at different growth stages of sorghum and millet cultivars grown in black plastic bags in glasshouse.

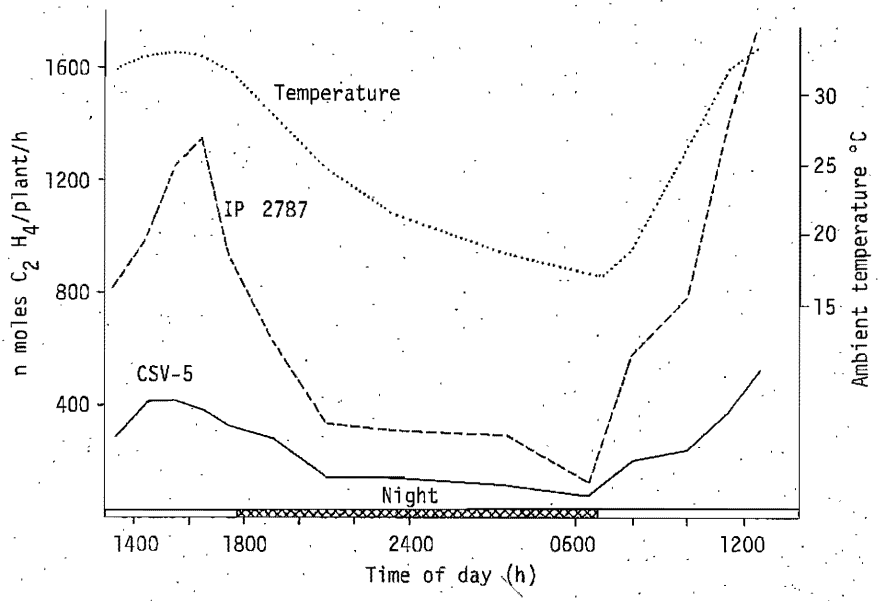

Fig. 3. Diurnal pattern of nitrogenase activity of intact sorghum and millet plants grown in pots in a glasshouse.

around the stem with silicone rubber and adding acetylene only to this root chamber (ICRISAT 1979). Activity increased during the day, being highest between 4 and 5 P.M., and declined markedly during the dark period. This pattern closely followed the ambient temperature, but our experiments on the effect of incubation temperature on nitrogenase activity of root-soil cores indicate only a two-fold increase as the temperature goes from 20^0 to 35^0, suggesting that much of the 13-fold variation in diurnal activity observed with intact plants is due to a factor other than temperature. It appears to be related to the photosynthetic activity of the plants. Diurnal variations in nitrogenase were also found for *Paspalum notatum* (Dobereiner and Day 1975), wheat (Dobereiner 1977) *Hyparrhenia rufa,* maize and *Loudetia simplex* (Balandreau et al. 1978).

Isotopic methods

Despite the sensitivity of the acetylene reduction assay, a much more promising approach is to use ^{15}N to label the available soil N pool and calculate N_2 fixation from the uptake of ^{15}N and the ratio of ^{15}N to ^{14}N in the dry matter of 'fixing' and 'non-fixing' plants (e.g. Rennie et al. 1978). This approach provides a measure of N_2 fixation integrated over time, something which the acetylene reduction assay cannot reliably do. Use of lightly labelled, well-equilibrated soils, where the ^{15}N:^{14}N ratio in mineralised N is virtually constant, would be a useful refinement for evaluation of N_2-fixing systems (D.H. Kohl and G.B. Shearer, personal communication).

Well-settled lysimeters, combined with addition of ^{15}N as labelled organic matter or fertiliser, are a very powerful system for establishing the nitrogen dynamics of the soil-plant system. The use of undisturbed soil cores in the lysimeter reduces the effects of soil disturbance on mineralisation and leaching (Dowdell and Webster 1980) and increases one's confidence in extrapolating the results to the field situation.

Soil processes at some sites have developed differences between the natural abundance of ^{15}N in the mineral N pool in the soil and the abundance in the atmosphere and it has been proposed that variations in abundance of ^{15}N between plants deriving some of their N from fixation, and those which are dependent on soil N can also be used to measure N_2 fixation (e.g. Kohl et al. 1980), but such studies have not been made on semi-arid tropic systems.

Because the rate of N_2 fixation is relatively low in associative symbiotic systems, long incubation times are needed if $^{15}N_2$ incorporation is to be studied; for intact plants, a fairly sophisticated gas lysimeter is required with control of humidity, temperature, CO_2 and O_2 levels (Witty and Day 1978). Uptake of N fixed in the root zone by the plant tops has been demonstrated using $^{15}N_2$ with the tropical grasses *Digitaria decumbens* and *Paspalum notatum* (De-Polli et al. 1977).

Chemical N Determination Methods

Nitrogen balances can also be measured for field crops by standard Kjeldahl

analyses, but more reliably for plants grown in pots in a defined volume of rooting medium, and inputs form N_2 fixation estimated. However, the relative insensitivity of the chemical methods for determination of N—either those based on the oxidative Dumas technique or on the Kjeldahl technique for wet digestion by sulphuric acid—means that only relatively large changes in the N content of the system can be reliably determined. In one season it is difficult to measure changes of less than 50 kg N/ha in total soil N. In pot experiments with soil containing 0.03% total N as the rooting medium, an error of 1% in the total N determination represents 15 mg N in the balance for 5 kg soil.

Host Genetic Variability for N_2 Fixation

Sorghum, millet and tropical grasses grown under low-fertility conditions show large differences in N uptake between varieties; some grass (Dobereiner 1977) sorghum (Pedersen et al. 1978) and millet varieties (Bouton et al. 1979) also show differences in nitrogenase activity. This suggests that it may be possible to select and breed for increased N_2 fixation. To examine this, we developed a soil root core assay for screening germplasm lines of millet and sorghum grown in the field for nitrogenase activity. With as little soil disturbance as possible, soil cores containing plant roots are taken in metal cylinders (15-cm diameter, 22-cm length) and incubated in sealed 6-litre plastic vessels under an atmosphere of *ca.* 15% acetylene in air. Gas samples are taken 17 and 24 hours later for gas chromatographic analysis of ethylene production.

Out of 284 pearl millet lines tested, 135 stimulated nitrogenase activity that was more than twice that of soil without plant roots. Eighteen lines stimulated high nitrogenase activity (equivalent to more than 100 μg N fixed/15 cm diam core/day). Two lines, GAM-73 and J-1407, were consistently active over several seasons (Table 8). Cultivars of the minor millets *Eleusine coracana, Panicum miliaceum, P. miliare, Panicum* sp., and *Setaria italica* also had high activity that was more consistent than for pearl millet.

Twenty-eight of 334 field-grown sorghum lines tested had high nitrogenase activity associated with their roots (equivalent to more than 100 μg N fixed/15 cm diam core/day). The active lines came from India (12 of 104 tested), West Africa (6 of 36), East and Central Africa (5 of 63), South Africa (6 of 29), USA (2 of 39), Thailand (1 of 2) and Japan (1 of 3). However, 167 lines stimulated nitrogenase activity of 25 μg N/core/per day, which was more than twice the mean activity of soil cores without plant roots (range 0-10 μg N/core/day). Some 15 lines have been consistently active in three or more seasons, though they are not consistently active on each assay occasion during the season (ICRISAT 1977).

Nitrogen fixation associated with both sorghum and millet was correlated with soil moisture, with increased activity in wet soil. Little activity was detected until plants were about 30 days old, with most consistent activity during the panicle emergence stage to the milky grain stage.

Experiments indicated that some of the plant-to-plant variability could be due to (1) disturbance of the soil-root interface during the coring process, (2) rough

handling during transport of the cores from field to laboratory for assay, (3) delay in adding acetylene to start the nitrogenase assay, (4) diurnal variation in nitrogenase activity and (5) variations in soil moisture content and temperature. A pot-culture technique has been developed for glasshouse-grown plants to reduce this variability. Intact plants can also be assayed by growing in a container in which the roots can be sealed off from the top of the plant.

Activity varies a great deal over seasons and from field to field. The large differences obtained in three Alfisol fields in the post-rainy season 1977 are plotted in Figure 4. Field P6 and the nursery were within 100 m of each other, but the nursery had received fertiliser at the rate of 60 kg N/ha at sowing, as against the other fields, which got only 20 kg N/ha. Least activity was found for plants grown in fields in which neither sorghum nor millet had recently grown, suggesting that the presence of the cereal may build up the soil population of N_2-fixing bacteria over time. If this is so, for crops grown in new fields, inoculation with N_2-fixing bacteria may enhance subsequent N_2 fixation associated with the crop.

A tube culture method for screening lines and bacterial cultures has also been developed. Nitrogenase activity has been detected within 10 days after sowing and there are significant differences between host-plant lines and between bacterial isolates in promoting this activity. We have yet to work out the correlation between the glasshouse-screening and field-screening methods. However, these new techniques of assaying intact plants offer great promise for screening lines and selecting plants with high activity, which can then be grown on to produce seed and/or used in a crossing programme. Preliminary observations with sorghum suggest that a hybrid will stimulate high nitrogenase activity if one of the parents has high activity.

N_2-Fixing Bacteria in Indian SAT Soils

The marked differences in nitrogenase activity of millet and sorghum between fields may be partly due to differences in their microbial populations, suggesting that it may be possible to obtain responses to seed inoculation with bacteria, as with legumes. There are many different genera and strains of bacteria which can be isolated from the soil and closely associated with the root, which can fix N_2, and the major problem in manipulating the crop-production system by inoculation is to choose a suitable inoculum strain or strains. The difficulty in studying the ecology of N_2-fixing bacteria is in devising selective media to count the populations of particular organisms. An added complication is the variable response between organism types to the partial pressure of oxygen in their vicinity.

Large populations of bacteria capable of growing in air on N-free media exist in some Alfisol fields at ICRISAT Center and there is also a marked (10-fold) rhizosphere effect (Table 9). A selection for particular types of bacteria also occurs in the root zone, resulting in less than half the types found in the bulk soil. The numbers of colony types and the population sizes varied with the carbon source in the isolation medium and were increased by adding a small amount of

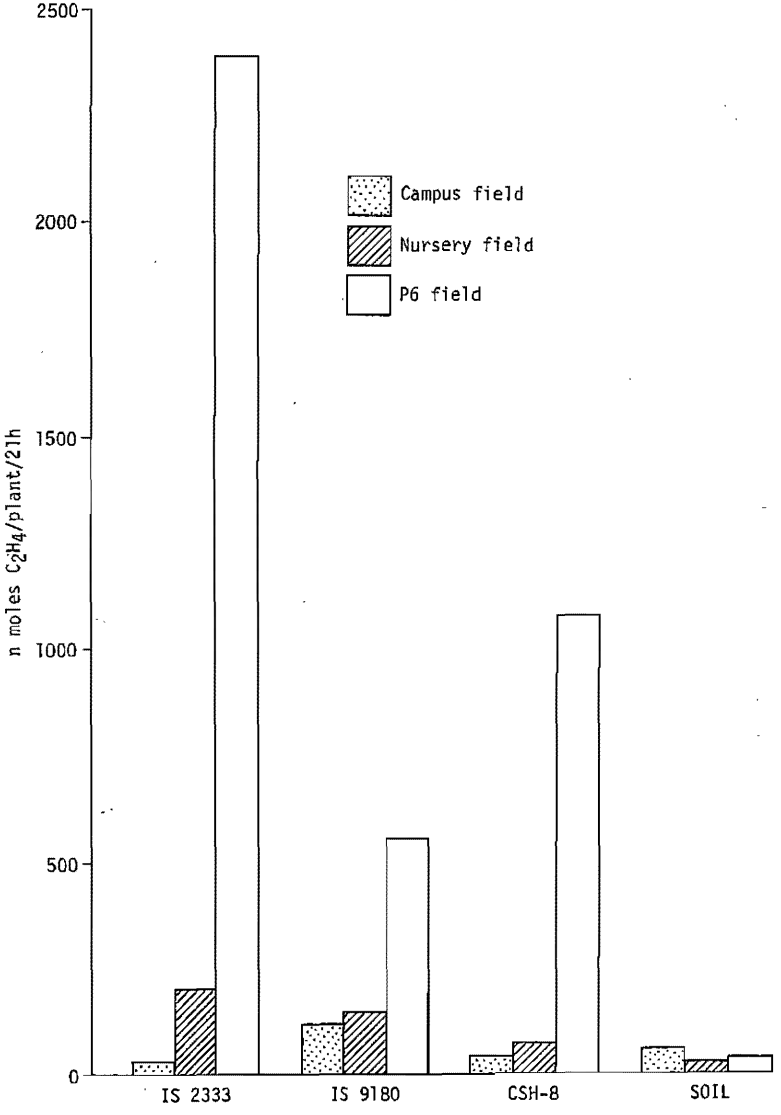

Fig. 4. Nitrogenase activity in three sorghum lines and non-cropped soils at three locations, ICRISAT Center, post-rainy season, 1979.

yeast extract (50 to 100 mg/litre) to the media. About 50% of the bacteria isolated because of their different colony characteristics were N_2-fixing bacteria. There were over a million Enterobacteriaceae/g soil, several species of which are known to fix N anaerobically.

For the cross between *Pennisetum purpureum* and *P. americanum* (Napier bajra line NB-21), some bacteria were very closely bound to the root and perhaps even in the root tissues. After shaking the root with glass beads to remove

surface-attached bacteria and then thoroughly sterilising the root surface with 1% chloramine T for 1 h, we recovered more than 400,000 presumptive N_2 fixers per g of fresh root from the root macerate (Table 9). Nitrogen-fixing isolates included both aerobic and anaerobic heterotrophs identified as *Enterobacter cloacae, Klebsiella pneumoniae, Azospirillum brasilense, Azospirillum lipoferum, Azospirillum* spp. and *Erwinia herbicola* which is generally thought of as a plant pathogen. Ten different organisms were isolated from the rhizosphere of related tropical grasses and, in addition to the above strains, included also *Bacillus polymyxa, Bacillus* spp., *Azotobacter vinelandii* and *Enterobacter* spp.

Table 9. Population of aerobic heterotrophs[a] in the rhizosphere and roots of Napier bajra.

Part treatment	No/g-fresh weight	Approximate no. of colony types
Rhizosphere	72.5×10^7	8
Rhizoplane	3.0×10^7	6
Rhizoplane (shaking with glass beads)	7.0×10^7	9
Roots (5 min. sterilisation)[b]	20.0×10^7	7
Roots (15 min. sterilisation)	7.0×10^7	7
Roots (1 h sterilisation)	4.0×10^5	7
Non-rhizosphere, 0-15 cm	8.0×10^7	17
Non-rhizosphere, 0-30 cm	6.5×10^7	18

a. Counted on N-free agar medium containing sucrose.
b. Sterilisation with 1% chloramine-T

We surveyed 200 sites in the traditional millet-growing areas in north-western India. The total population of organisms growing on a nitrogen-free sucrose medium supplemented with 50 mg/litre yeast extract (YE) ranged from 10^6 to 10^7/g soil. Nitrogenase activity was detected in 45% of the 3700 isolates made from the highest dilution plates. Every soil contained organisms which produced pellicles and reduced acetylene on a malate medium (Dobereiner 1980), with a most probable number (MPN) of N_2 fixers varying from 10^3 to 10^4 per g soil. Some of these soils did not contain *Azospirillum,* and Enterobacteriaceae and Pseudomonads were commonly isolated from the malate enrichment cultures. The isolates from the sucrose-based medium can be classified into at least seven different genera of N_2-fixing bacteria, including types believed to be rare, which we are still identifying. *Enterobacter cloacae* was the most common isolate. Some *Pseudomonas* types were also nitrogenase positive. There were at least 10^6 actinomycete-like organisms per g soil in every sample and of the 229 isolates, 70 had nitrogenase activity on sucrose + YE medium. We are not certain of the purity of these isolates at present, as it is often extremely difficult to free the cultures of small contaminating rods and yeast-like organisms, particularly if the culture produces a large amount of capsular material or other extracellular polysaccharide.

From our attempts to identify the organisms we feel that there is often a

continuum of types overlapping in properties between named genera and species. The isolates obtained from this study could be classified on colony morphology into at least 22 different groups. However, they represent only the most numerous organisms able to grow on two media. Use of the 'spermosphere model' (J. Balandreau personal communication)—where sterile plants are grown in agar in the dark and inoculated with soil dilutions, followed by isolation of bacteria from nitrogenase-positive tubes—may give more information on the selection and stimulation of organisms in the rhizosphere of different species.

Response to Inoculation

In field experiments in Israel, inoculation with *Azospirillum brasilense* has substantially increased plant growth and yield of sorghum, maize and the millets *Setaria italica* and *Panicum miliaceum* (Kapulnik *et al.* 1981). The crops were grown in the summer under irrigation, high irradiation and soil temperatures, and relatively high soil nitrate levels. Pot experiments indicated that inoculation increased plant N uptake of maize and *Setaria,* presumed to be from N_2 fixation (Nur *et al.* 1980; Cohen *et al.* 1980). Smith *et al.* (1976) obtained an increase in dry-matter yield of pearl millet grown in the field at Florida and given 40 and 80 kg N/ha fertiliser, following inoculation with *Azospirillum lipoferum.* Other inoculation experiments with sorghum (Barber *et al.* 1976; Pedersen *et al.* 1978; Klucas and Pedersen 1980), pearl millet, (Barber *et al.* 1979; Bouton *et al.* 1979) and *Cynodon dactylon* (Baltensperger *et al.* 1978) in North America have given small or no response (see Boddey and Dobereiner, this symposium, for fuller discussion). One unresolved question is whether the *Azospirillum* inoculation produces its response through hormonal effects on plant growth, or through N_2 fixation, or through both (Tien *et al.* 1979), as total N balances have not been determined in these experiments.

In our pot culture studies, the response of sorghum and millet to inoculation with N_2-fixing bacteria varied with the growth medium and amount of N fertiliser added. Table 10 shows the response of sorghum grown in pots in non-sterile Alfisol soil, to inoculation with *Azospirillum lipoferum,* as well as to a

Table 10. Effect of inoculation with N_2-fixing bacteria on sorghum grain and dry-matter production.

Culture	Grainweight/plot (g)[a]			Total dry matter/plot (g)[a]		
	0 N	20 N	40 N	0 N	20 N	40 N
Azospirillum lipoferum	10.0	13.1	18.9	66.3	79.8*	93.5
Azotobacter chroococcum	9.3	10.9	15.9	58.5	74.0	93.7
Napier bajra root extract	10.0	12.8	22.0*	62.2*	81.5*	96.1
Uninoculated broth	7.1	9.9	17.9	48.1	67.2	85.4
L.S.D.*($P<0.05$)		3.7			12.6	
C.V		19.8			11.6	

a. Average of four replications. In each pot four plants were grown in unsterilised Alfisol in a glasshouse for 103 days.

crude Napier bajra root extract, where grain yield was increased by up to 22% and total dry-matter production by up to 29%. The interesting aspect of this experiment is that the yield increase occurred even when the equivalent of 40 kg N/ha was added. Variability in response between replicates is a major problem in this kind of experimentation. High coefficients of variation mean that only large responses are statistically significant. In our work we are now increasing the replication to help reduce the uncertainty.

Conclusion

The origin of the N for cereal crop production in the SAT still remains somewhat of an enigma. Similar levels of soil N seem to accumulate in soil under certain grass fallows as under grain legumes. Is this demonstrating the superior ability of the perennial grasses to exploit the nitrate leached to depths in the soil beyond the reach of many annual species and/or the result of nitrogen fixation? Indeed we do not know the relative importance of different soil layers in providing the N for cereal crop growth. The millets, sorghum and many grasses adapted to the SAT support root-associated nitrogenase activity. The problem is to quantify this to assess its importance for the N cycle of SAT soils. Long-term experiments to measure the N balances of the soil-plant system should indicate the relative order of magnitude of any N_2 fixation. Although nitrogenase activity occurs in the root zone of many plants, a key issue to be resolved is the efficiency of uptake by the plant of the N fixed, and the effect of competition by other organisms in the rhizosphere on this. More use should be made of ^{15}N, despite the relatively high costs involved. We must have definitive answers to questions concerning the level of N_2 fixation, its interaction with soil and fertiliser mineral N and the differences between plant genotypes in stimulating fixation.

Prospects for increasing this N_2 fixation by selection and breeding of the host plant, by inoculating seed with N_2-fixing bacteria and by soil management practices are good enough to justify a well-funded, concerted research effort. It is important to remember that there is a large environmental component in non-symbiotic N_2 fixation in the SAT, with activity stimulated by high irradiation levels, ambient temperatures and soil moisture, and with large effects of soil type, indicating the need to conduct much of the necessary research in the semi-arid tropics for meaningful results. If non-symbiotic N fixation can be increased over the present level by only 10 kg N/ha per crop, this represents a potential fertiliser saving of 860,000 tonnes of N—worth about $53.3 million at present prices paid by the Indian farmers.

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