THE EFFECT OF EDAPHIC FACTORS ON N_2 FIXATION WITH SPECIAL EMPHASIS ON OGANIC MATTER IN SOILS

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Although the state of our knowledge of the influence of edaphic factors on legume-*Rhizobium* symbiosis is relatively well advanced, many questions remain unanswered about the role of organic soil matter (cf. Shina, 1977) and we have very little information about the influence of edaphic factors on rhizospheric and asymbiotic N_2 fixation.

In this paper three categories of processes will be considered:

(1) symbiotic N_2 fixation, restricted to the case of legume-*Rhizobium* symbiosis and to that of the *Azolla-Anabaena* symbiosis;

(2) rizospheric N_2 fixation, i.e., N_2 fixation by diazotrophs associated with the root system (either living, drying or dead roots) ; and

(3) asymbiotic N_2 fixation, i.e., N_2 fixation by free-living diazotrophs, either bacteria or blue-green algae.

The aim of this review is to draw attention to aspects of the ecology of N_2 fixation hitherto overlooked and to suggest steps necessary to increase this beneficial microbial activity through better soil management.

Although we are particularly concerned here with edaphic factors, one should keep in mind the fact that N_2 fixation by a given system or organism also depends upon climatic conditions, especially light.

1. Edaphic factors other than organic matter.

1.1. Edaphic factors affecting the *Rhizobium*-legume symbiosis

Since the influence of edaphic factors other than organic matter on the

Rhizobium-legume symbiosis has been recently reviewed by Gibson (1977), we shall give here only a brief account of our present knowledge about this problem, restricting the discussion mostly to the case of tropical legumes.

1.1.1. Physical factors.

Soil temperature in known to have marked effects on nodulation and nitrogen fixation. In tropical soils nodulation is adversly affected by the high temperatures often encountered in semi-arid or arid soils. Thus nitrogen fixation by chick-pea is inhibited when temperatures rise to 33°C during the day (Dart *et al.*, 1975).

Some attention has been given recently to the influence of moisture stresses on the *Rhizobium*-legume symbiosis: deficiency and excess are both detrimental. Nodulation does not occur when the soil is too dry (Beadle, 1964, Diatloff, 1968). Such was the case for peanut in central Senegal in 1977. Nodulation was delayed by drought up to the 50th day after sowing so that N_2 fixation (measured by the ARA, acetylene reducing activity) started only after that date (Fig. 1...). Even when plants are well nodulated, N_2 fixation can be severely depressed by a moisture stress occurring after most nodules have been formed (Sprent, 1972). This situation occurred in the case of peanut in central Senegal in 1976: a drought that occurred in the middle of the growth cycle drastically reduced measured ARA (Fig. 1.). Recent studies of soybean have shown that N_2 fixation appeared to "be affected only slightly by the moisture content of soil around the nodules, if plants received adequate moisture from below the nodule zone" (Hume *et al.* 1976). Soil moisture deficits are often associated with high soil temperatures, so that it is difficult to infer from field studies the respective role of moisture deficit and temperature excess. Such conditions may limit the areas and seasons in which legumes can be grown (Dart, 1976).Waterlogging, especially when associated with poor soil structure, is responsible for low oxygen levels in soils. The general topic of the effect of the gaseous environment on nodulation and symbiotic function was discussed in detail by Pate (1975), Minchin and Pate (1975), and Criswell *et al.* (1977). Here it will be sufficient to recall that waterlogging drastically reduces nodulation and N_2 fixation (e.g., Diatloff, 1967). The relatively high O_2 requirements of the nodules may explain the following conclusion of experiments carried out in Israel on the inoculation of irrigated peanut during the hot and dry season; "the placement of the inoculum at a depth of 12cm resulted in poorer nodulation than at a depth of 3-4cm, despite the higher soil moisture at the greater depth" (Shimshi *et al.*, 1967).

1.1.2. Chemical factors

Much is known about the nutrients required for the legume, the *Rhizobium* and for the legume-*Rhizobium* association itself. These aspects and the effect of soil acidity have been reviewed in detail elsewhere, (Quispel, 1974; Hardy and Gibson, 1977). We shall only recall here that nodulation is mostly affected by calcium, boron, acidity and, to a lesser extents phosphorus and sulphur.

The functioning of the symbiosis is mostly dependent upon molybdenum, sulphur, calcium and boron (Dart, 1976). Soil acidity, which is prevalent in tropical situations, is generally associated with low Ca, P and Mo contents and high Al and eventually Mn contents. Though tropical legumes in their nodulation and nitrogen fixation are generally more tolerant to low soil pH than temperate legumes, liming is often useful, together with application of Mo if this element is deficient (e.g., Day and Franco, 1976). Groundnut in its nodulation and nitrogen fixation is relatively tolerant to acid pH and high aluminium levels (Dart, 1976). Excessive pH (>8,0) resulting from irrigation or ashes originating from burning crop residues markedly limit peanut nodulation (Ganry, 1977; Dreyfus, 1977, personal communications). Since legumes require relatively large amounts of phosphorus , except when they are associated with vescicular-arbuscular mycorrhizae (Mosse *et al.*,1976), application of P fertilizers is nearly always recommended in tropical conditions.

Soluble combined nitrogen is known to decrease nodulation of legumes (cf. Dart's review, 1974). However, nitrate induced inhibition is not so well established (Gibson, 1977). On the other hand, N_2 fixation is stimulated by small amounts of combined N (starter nitrogen) applied before the nodules are formed, i.e., during a phase of nitrogen hunger. Later the effect of combined nitrogen is obviously depressive. The relationships between total plant nitrogen during a growing season has been diagrammed by Gibson (1977) (Fig. 2). The use of fertilizers compatible with N_2 fixation has been suggested by Hardy *et al.*,(1973) as a means of overcoming this inhibiting effect of conventional nitrogen fertilizers (Table 1).

1.1.3. Biological factors.

Among the edaphic factors, biological factors are sometimes overlooked. Such an omission may obscure the interpretation of environmental studies. Biological factors include non-pathogenic microorganisms (e.g., mycorrhizae, saprophitic bacteria) or pathogenic organisms (viruses, insects, nematodes) that affect nodulation and N_2 fixation.

The adverse effect of antagonistic microorganisms was often reported to reduce nodulation (cf. Dart, 1974, for reference list; Kumara Rao *et al.*, 1974). Practical means of overcoming this antagonism are urgently needed in some semi-arid soils. Competition between *Rhizobium* strains is another difficult problem, which has hitherto received no practical solution. Nematode infection has been reported to inhibit nodulation by some unknown process; nematodes can also invade the nodule (see Gibson, 1977, for reference list; Germani and Gautreau, 1976). Application of nematicides restores nodulation and consequently N_2 fixation. However, unfavorable aftereffects of nematicides on N_2 fixation have been reported, resulting from a desequilibrium of the nematode populations, more virulent species being favored by nematicide applied the previous year (Germani, 1977, personal communication). Such experimental data suggest that more elaborated methods of pest control are urgently needed in order to maintain the legume potential of N_2 fixation. Recently attention has been given to the effect of endomycorrhizae on the legume-*Rhizobium* symbiosis: endomycorrhizae do not only substantially increase P uptake by the plant but also lower the host root's resistence to water transport, probably by improving the nutrient uptake (Safir *et al.*,1972). Endomycorrhizae improve both nodulation and N_2 fixation (Mosse *et al.*,1976; Dart, 1976). Suitable mycorrhizal inoculation together with application of some form of organic matter and phosphorus fertilizer should allow the extension of legume crops in poor tropical soils. However, more research is needed in order to produce endomycorrhizal inoculants on a proper scale.

Development of legume crops is often adversely affected by different pathogens and insects which impede nodule development and symbiotic N_2 fixation. Such limiting factors should not be overlooked, especially in stressed conditions.

1.2. Edaphic factors affecting rhizospheric and symbiotic N_2 fixation

Relatively little attention has been given to the effect of edaphic factors on these processes. But growing interest in rhizospheric N_2 fixation, investigations are currently underway to elucidate the role of the main limiting edaphic factors.

1.2.1. Physical factors.

Contrary to symbiotic N_2 fixation, rhizospheric and asymbiotic fixation are markedly promoted by waterlogging (e.g. Dommergues *et al.*, 1972), which explains why high rates of rhizospheric N_2 fixation are associated with plants in waterlogged habitats: rice (e.g. Balandreau *et al.*, 1976; Yoshida, 1971, 1972) *Juncus* (Tjepkema and Evans, 1976), marine grasses such as *Thalassia* (Patriquin and Knowles, 1972), *Avicennia* sp, *Rhizophora* sp (Silver *et al.*, 1976). These results are perfectly consistent with the well known sensitivity of the nitrogenase system and pure culture of diazotrophs, even aerobic ones (see Knowles, 1976, for reference list). Conversely, moisture deficits severely depress rhizospheric

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N_2 fixation. As far as moisture is concerned, the behaviour of N_2 fixing algae is parallel to that of diazotrophs associated with plant roots.

Temperature is also a key factor which acts directly upon the diazotroph or through the plant (rhizospheric diazotrophs).; low temperatures were shown to limit rhizospheric fixation (Balandreau *et al.*, 1977)

1.2.2. Chemical factors

Soluble combined nitrogen inhibits rhizospheric N_2 fixation. However, preliminary data (Rinaudo and Ganry, personal communications) indicate that starter doses of nitrogen would be beneficial. On the other hand field experiments set up by Smith *et al.*, 1976, suggest that application of medium rates of nitrogen fertilizers would stimulate rhizospheric N_2 fixation. In a fertility trial with lowland rice, nitrogen dressing (140 kg N ha^{-1}) inhibited nitrogen fixation only temporarily. Since rice plants rapidly absorb ammonium nitrogen, nitrogen repression is restricted to the first stages of growth (Trolldenier, 1977). The critical threshold apparently varies widely with the soil type and the plant.

1.2.3. Biological factors

Diem *et al.*, (1977) indicated that actinomycetes may account for the limited development of a *Beijerinckia* strain in an alluvial paddy soil of Camargue, suggesting that antagonists may eliminate diazotrophs from certain habitats.

2. Influence of organic matter on N_2 fixation.

2.1. Influence of organic matter on symbiotic N_2 fixation.

The overall effect of organic matter on N_2 fixation by legumes was reported to be generally, but not always, beneficial. Most examples given here are related to incorporation of crop residues, animal manure, especially farm yard manure (FYM), compost and some other compounds. From pot experiments wheat straw incorporati (3 and 6 $t.ha^{-1}$) was shown to increase the yield of soybean from 0.871 (control) to 0.925 and 1.022g, and the total protein content of seeds from 273mg (control) respectively to 301 and 358mg (Shivashankar *et al.*, 1976). This effect of wheat straw incorporation was attributed to CO_2 enrichment of the lower atmosphere, which is known to improve N_2 fixation (Havelka and Hardy, 1976). Addition of soybean meal at 135Kg $N. ha^{-1}$, increased the yield of field grown soybean by 23% and N_2 (C_2H_2) fixation by 12%, suggesting that soybean meal acted as a source of nitrogen compatible with N_2 fixation, wheareas urea or NH_4^+ application inhibited nitrogen fixation (Table 1).

Mulder and Van Veen (1960) reported that extracts of stable manure "not only promoted multiplication of *Rhizobium trifolii* infection of the roots but also the growth of the nodules". This stimulative effect was the same as that observed with yeast extract and sterilized *Rhizobium* cells. No explanation of the observed effect was proposed.

Table 1. INCORPORATION INTO THE SOIL OF SOYBEAN MEAL, UREA AND AMMONIUM FERTILIZER AND ITS EFFECTS ON THE N_2 FIXATION AND YIELD OF FIELD GROWN SOYBEAN (Hardy *et al.*, 1973)

Application	N_2 (C_2H_2) fixation	Yield
Soybean meal	112	123
Urea	48	109
NH_4^+ fertilizer	51	104

Data expressed as a percentage of control.

Saint-Macary (personal communication, 1977) studied the effect of the addition of different forms of organic matter, compared with urea and urea-formaldehy on nodulation and N_2 fixation, in soybean plants, (cv. Jupiter), grown in the open in pots in a sandy Senegalese (Dior) soil. Additions expressed in terms of nitrogen were made at the rate of 30 and 60kg $N.ha^{-1}$. Table 2 indicates that application of animal manure (60Kg $N. ha^{-1}$) did not affect nodulation and increased (slightly, though non significantly), N_2 fixation measured by Acetylene Reducing Activity (ARA), suggesting that the animal manure used in his experiment behaved like a compatible fertilizer. Contrary to wheat straw pearl, millet straw appeared to inhibit nodulation and ARA. This discrepancy cannot be attributed to the rate of application of straw (9-18g/pot of 4kg in Shivashankar's experiment; 4-8g/pot of 3Kg in Saint-Macary's experiment) but could possibly result from the relatively high concentration of phenolic compounds which are present in pearl millet straw (Ganry *et al.*, 1978). Application of animal manure to field grown peanut in Senegal was shown to increase ARA by ca. 50% (Ganry et Wey, personal communication). Preliminary observations suggest that green manure could significantly depress N_2 fixation in peanut (Ganry, personål communication). Few experiments were carried out using somewhat purified humic compounds such as humic acid or fulvic acid.

Aftereffects of different types of organic matter on N_2 fixation in pot-grown peanuts were estimated by Ganry *et al.*, using two methods; (1) calculation of N_2 fixation from dilution of ^{15}N derived from the soil in the N_2 fixing plant (Legg and Sloger, 1977); (2) determining the difference between the total N content in peanuts and total N in a non-N_2 fixing plant taking up the same amount of soil nitrogen. Compost and FYM markedly favored N_2 fixation, which was increased by 30-50%. On the other hand, pearl millet straw had no effect (Table 3).

The latter result seems to be inconsistent with results reported in Table 2. The discrepancy can be attributed to the fact that in one case (Table 2) straw was incorporated a short time before the legume was sown and in the other case (Table 3) straw had been incorporated the previous year.

Preliminary observations suggest that green manure could significantly depress N_2 fixation in peanuts (Ganry, personal communication).
Few experiments were carried out, using somewhat purified humic compounds such as humic or fulvic acid. Favorable effects on the *Trifolium-Rhizobium* symbiosis were reported by Bhardwaj and Gaur (1968) and Myskow (1970).

Table 3. AFTEREFFECTS OF DIFFERENT TYPES OF ORGANIC MATTER ON N_2 FIXATION IN PEANUTS, EXPRESSED IN mg OF N_2 FIXED PER PLANT.
(Ganry, Guiraud and Dommergues, unpublished)

Treatments	Estimation of N_2 fixation by 2 methods	
	^{15}N	Difference
Control	101	97
Incorporation of Pearl millet straw	111	101
Incorporation of compost	142	158
Incorporation FYM	136	146

2.2. Influence of organic matter on rhizospheric N_2 fixation.

Comparisons of rhizospheric N_2 fixation carried out with the same rice cultivar in different soil types have shown that this biological process depends critically on the soil type. Thus Rinaudo *et al.*, 1971 reported large variations in the Acetylene Reducing Activity (ARA) of the rizosphere of IR8 rice seedlings grown in 3 differents soils from the Ivory Coast (Table 4). Obviously such variations are not related to the organic matter content of the soil. **Moreover,** in contrast with the established fact that combined nitrogen inhibits the activity of diazotrophs the highest ARA occurred in the soil with the highest total nitrogen content. This result suggests that some forms of organic soil nitrogen could probably be compatible with N_2 fixation.

Table 4. ACETYLENE REDUCING ACTIVITY (ARA) OF THE RHIZOSPHERE OF 20 DAY-OLD IR8 RICE SEEDLINGS GROWN IN 3 SOILS FROM THE IVORY COAST (Rinaudo *et al.*, 1971).

Soils	Texture (percentage) Clay	Silt	Sand	Total C content (10^{-3})	Total N content (10^{-3})	C/N	pH	ARA
Dabou	63	32	1.5	46.95	3.00	15.6	6.2	33
Abengourou	30	38	28	31.75	2.57	12.8	6.5	9
Yamoussokro	27	28	44	17.80	1.51	11.8	6.9	119

ARA expressed in terms of nanomoles C_2H_4 per g dry rhizosphere soil per hour.

2.3. Influence of organic matter additions on symbiotic fixation in the soil.

Adding organic energy sources, especially carbohydrates such as glucose or starch, to soil is well known to promote asymbiotic nitrogen fixation, provided no limiting factor (e.g., phosphorus deficiency) impedes the activity of the diazotrophic microorganisms. When artificial energy sources like sugar are replaced by natural plant residues, N_2 fixation occurs only if the type of organic matter used has a high C/N ratio and is easily decomposed.

High nitrogen fixation rates obtained in the laboratory by incorporating such organic energy sources have seldom been reported in the field because the amount of organic matter incorporated in such conditions is usually much lower (0.1% instead of 1%) in order to avoid the depletion of organic nitrogen which would be detrimental to the plant. One should add that it is difficult to evaluate N balances in the field because of the magnitude of sampling errors and the occurrence of uncontrollable factors. Despite these limitations, Abd-el Malek (1971) obtained substantial nitrogen gain by incorporating additional plant residues into different Egyptian soils, which are characterized by their low organic content and their richness in calcium carbonate. Unfortunately, no data were given regarding the plant yields.

A study carried out in Senegal by Beye (1977) showed that ploughing straw into waterlogged paddy soil ("sol gris") significantly increased the yield of paddy and the N content of the rice plants, which suggests that the incorporated straw had stimulated nitrogen fixation and subsequently increased the amount of nitrogen available to the plant. This favorable effect on rice fields was not found when experiments were performed using a different soil type (sandy paddy soil). This discrepancy could be attributed to the fact that the latter soil type harbours a much less active N_2 fixing microflora (Diem, personal communication). In the soil of the IRRI Research Station, Watanabe (1975) has found that the N_2 fixing-activity was markedly enhanced in leftover rice stubbles.

Studies reported above are incomplete since they were carried out either by microbiologists or by soil scientists and not by interdisciplinary teams, but they suggest that the incorporation of organic matter stimulates N_2 fixation and could possibly increase the crop yield in some soil types (e.g., Senegalese "sol gris"). Further investigations are needed in order to check the yield increase is really due to N_2 fixed at the expense of these residues, acting as a source of energy for soil diazotrophs.

2.4. *Azolla* and blue-green algae utilization in rice culture.

The agronomic significance of the *Azolla-Anabaenae* association, which has been reviewed by More (1969) and more recently by Silvester (1977), is now well known. Pot experiments in Indonesia showed that inoculating rice with *Azolla* markedly increased rice production and suggested that fixed nitrogen was absorbed by rice after the death of *Azolla* (Table 5).

Table 5. EFFECT OF <u>AZOLLA</u> ON GROWTH AND NITROGEN CONTENT OF RICE PLANTS WITH AND WITHOUT A 4 DAY DRY PERIOD IN WHICH <u>AZOLLA</u> PLANTS DIED (Silvester, 1977)

Treatment	Water supply	Rice Production (per pot)	
		Dry wt. (g)	Total N (mg)
Control minus *Azolla*	Continuous	36.9	221
	Dry period	40.0	240
Half *Azolla*	Continuous	51.5	301
	Dry period	51.0	309
Full *Azolla*	Continuous	43.3	316
	Dry period	48.9	465

At the present time, *Azolla* is often used as a green manure. It is introduced into the paddy field, allowed to grow and cover the water surface, then ploughed in when it is dead. *Azolla* may be grown at the same time as rice or in the interval between two rice crops or a dry, irrigated crop. Recent field trials carried out at IRRI's station in Los Banos showed that "*Azolla* that grown in field plots for 20 to 23 days accumulated 24Kg N per ha. From October to January (106 days) five crops of *Azolla* were harvested, accumulating 120Kg N/ha" (Watanabe, 1977).

Inoculating a plot with *Azolla* at the time of transplanting resulted in an increase in grain yield of 13% over a control plot (Watanabe, 1977). This experiment and other trials suggest that the beneficial effect of *Azolla* may be do not only to the release of nitrogen in the soil but also to other processes which are largely unknown. Thus Kulasooriya and de Silva (1977) found that *Azolla* treatment outdid the urea treatment in percentage of filled grains. "*Azolla*-plus-fertilizer registered 132% as many filled grains per panicle as the control; the urea treatment registered 95%, which indicates that *Azolla* could act by reducing sterility". Since optimum growth conditions of *Azolla* are not very well known, further studies are needed in order to solve the problems regarding *Azolla* inoculating of paddy fields on a large scale. One must keep in mind the fact that limiting factors that cannot be controlled, may impede this application. Thus temperatures above 31°C are known to inhibit the growth of *Azolla* (Watanabe, 1977), restricting its use to some climatic conditions.

Inoculating rice with blue-green algae has been done extensively in Japan and in India. In India algal inoculation was shown to have a significant positive effect on rice yields even with high rates of fertilization (Venkataraman, 1972). The development of algalization is desirable, but it may be limited, as is *Azolla* inoculation, by uncontrollable environmental conditions. Thus Roger and Raynaud (1976) have shown that high light intensities, which are often encountered in semi-arid situations such as in Senegal, may inhibit the growth and nitrogen fixing activity of blue-green algae.

2.5. Promoting N_2 fixation in compost.

During the composting process of plant residues or various objectional wastes, nitrogen losses occur through denitrification and leaching. The question arises wheter one could balance such losses by promoting N_2 fixation at a given stage of the decomposition process. Exploring such a possibility is most desirable especially in situations where nitrogen fertilizers are expensive.

3. Conclusion.

It is very difficult to discuss the effect of organic soil matter on biological N_2 fixation for at least 3 reasons:

- organic matter is not a definite compound but is made up of a host of different heterogeneous materials comprising mostly plant residues and their decomposition and re-synthesis products;
- the effect of organic matter depends critically on actual environmental conditions;
- organic matter may directly and/or indirectly affect N_2 fixation process.

Discrepancies in results reported in the literature may be attributed to the heterogeneity of the composition of organic matter. An example has already been given which is related to the effect of straw. Table 6 shows the main hypotheses which could explain most of the direct or indirect effects of organic matter on N_2 fixation.

Table 6. HYPOTHETICAL MECHANISMS BY WHICH SOIL ORGANIC MATTER CAN DIRECTLY OR INDIRECTLY AFFECT N_2 FIXATION.

Hypothesis	Organic matter as	direct effect	indirect effect through the plant
1.	Source of antimicrobial compounds or growth factors for microorganisms	+	
2.	Factor of stimulation or inhibition of plant growth		+
3.	Agent of improvement of physical soil properties:microstructure, aggregation, water storage	+	+
4.	Source of organic or inorganic nutrients (especially nitrogen)	+	++
5.	Buffer, ion exchange material agent of prevention of phosphate fixation	+	+

First organic matter can have a direct and specific effect on diazotrophs acting as a source of antimicrobial compounds or as a source of growth factors (hypothesis 1).

Second organic matter can also have a specific effect on plants, organic commpound "being taken up by the plants through the roots and participating in in the plant's metabolism" (Flaig, 1972), thus promoting or inhibiting plant growth (hypothesis 2).

Third organic matter, specially in tropical conditions, plays a major role in improving physical and chemical soil properties, thus affecting the plant and associated diazotrophs (symbiotic and rhizospheric N_2 fixation) or the diazotrophs alone (asymbiotic N_2 fixation) (hypothesis 3-4-5). In semi-arid soils, the favorable effect of organic matter on the water retention characteristics of soils (Charreau, 1974) should theorically enhance N_2 fixation, especially during drought spells since soil humidity is one of the major limiting factors for N_2 fixation (cf. par. I.I.I.). A good soil aeration is a prerequisite for symbiotic N_2 fixation improvement of soil structure should result in an increase of N_2 fixation. Likewise, improvement of chemical properties such as the prevention of phosphorus fixation by organic matter (Charreau, 1974) should also favor N_2 fixation processes.

Effects of organic matter on N_2 fixation have not been clearly demonstrated up till now. The reason is that the problem is highly complex. Such complexity has obviously rebuffed most researchers. However, in spite of such difficulties, research in that field should be developped because agronomic observations and preliminary experimental data clearly suggest that organic matter plays a prominent role in the expression of the N_2 fixing ability of the different systems. Organic matter management seems a promising means of directly or indirectly stimulating

N_2 fixation, thus saving nitrogen fertilizers.

Recent advances in the field of soil microbiology and in that of modelling biological processes (cf. Schmidt *et al.*, 1977) will probably facilitate the development of such urgently needed research. However, one must bear in mind the fact that these investigations should always be carried out by multidisciplinary teams involving not only soil microbiologist, but also other groups of soil scientists, plant physiologist and, of course, agronomists.

Captions for figures

Fig. 1. Age: nodule dry weight and age: N_2 (C_2H_2) fixing activity of field grown peanuts over the first part of two growing season.
For each year drought spells are indicated by interrupted lines.
The 1976 drought lasted from 35th up to 50th day; the 1977 drought lasted from 20th up to 38th day. (Ducerf, unpublished).

Fig. 2. Diagramatic representation of the effects of combined nitrogen (soil plus fertilizer) on the nitrogen assimilation by legumes over a growing season. (Gibson, 1977).

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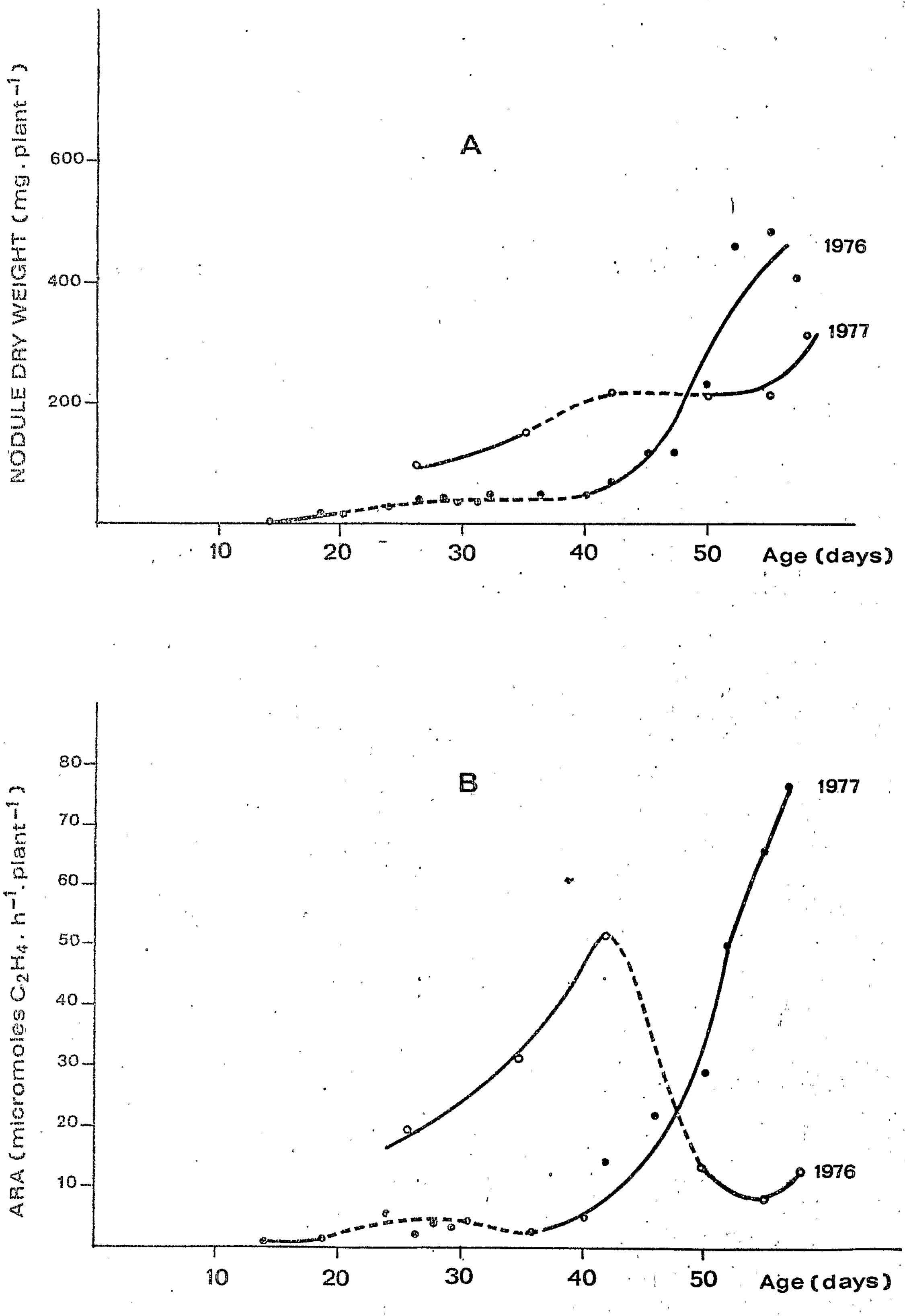
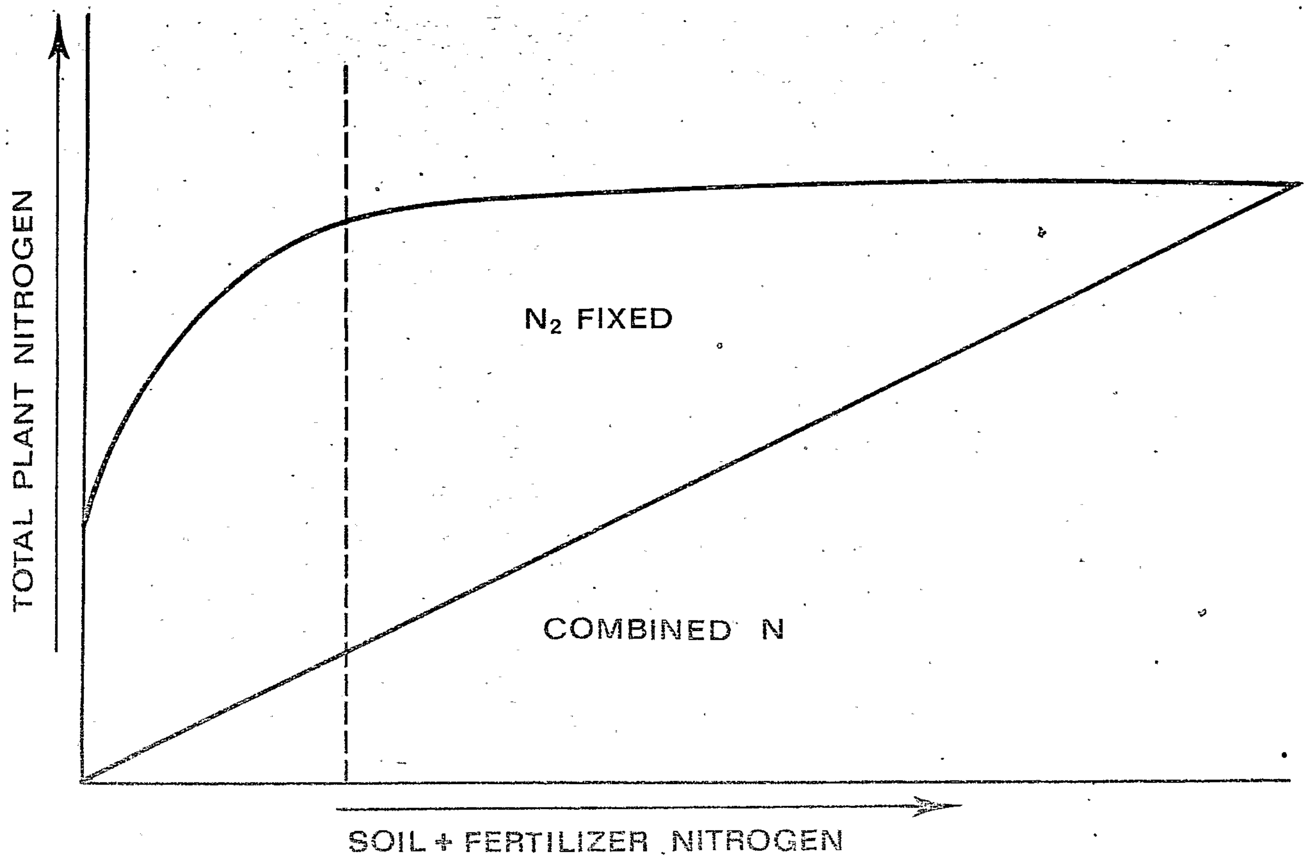

A
NODULE DRY WEIGHT (mg . plant^{-1})
600
400
200
1976
1977
10
20
30
40
50
Age (days)
B
ARA (micromoles C_2H_4 . h^{-1} . plant^{-1})
80
70
60
50
40
30
20
10
1977
1976
10
20
30
40
50
Age (days)


TOTAL PLANT NITROGEN
N_2 FIXED
COMBINED N
SOIL + FERTILIZER NITROGEN