Associative nitrogen fixation and growth of maize in a Brazilian rainforest soil as affected by *Azospirillum* and organic materials

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Abstract

A greenhouse experiment was carried out to evaluate the effects of applying organic materials and inoculation with *Azospirillum* sp. on dry matter production and nitrogen fixation of maize (*Zea mays)* grown in a Podzol Hydromorphic soil of the rainforest zone of Pernambuco, north-east Brazil. The treatments consisted of the addition of organic materials of different origin (cow manure, solid vinasse, vermicompost and fruit-horticultural compost) applied at levels of 0, 10 and 20 t/ha. Strain NFB 2, isolated from maize at the University Federal Rural of Pernambuco, and strain Sp 242, isolated from wheat at the Agrobiology Research Center — EMBRAPA, were applied individually to maize seedlings, and a control treatment without *Azospirillum* inoculation was included. Plants were cropped at 50 days after seedling transplant. *Azospirillum* inoculation as strain NFB 2 reduced total N accumulation, in the presence of applied organic material. Organic material addition increased the nitrogenase activity in the roots, and the fruit-horticultural compost (wider C:N ratio) showed the most pronounced effect on this parameter. The organic materials increased maize growth with the best responses at a rate of 20 t/ha.

Index terms: Diazotrophics, C:N ratio, organic matters, cow manure, vermicompost, vinasse.

Introduction

Cereals and legumes represent the largest food source in the modern world, especially in developing countries and maize (*Zea mays*) is the cereal of highest consumption, for both humans and animals. Maize production has been limited by the onerous inputs required, especially of nitrogen fertilisers. Associative biological nitrogen fixation is the most important alternative technique for supplying this nutrient to plants and can mean increases in productivity as well as a reduction in fertiliser costs. The Council of Researches of the American Government (NRC) recommended investment in research on biological fixation of nitrogen as a means of reducing costs of nitrogen fertiliser required and to minimise environmental problems caused by the use of fertilisers especially nitrogenous compounds (Hardy and Eaglesham 1995). Application of organic matter is usually recommended in intensive agriculture for amendment of the soil reaction, to increase water-holding capacity, for aeration and to increase microbial activity.

Roots of maize and other grasses are colonised by diazotrophic bacteria particularly *Azospirillum*, that have been the subject of ecological, physiological and genetic studies as well as studies on the processes of microbial colonisation (Baldani *et al*. 1997). These studies examined practical aspects of diazotrophic inoculation (Baldani *et al*. 1999) and the interaction with different kinds of organic compounds used in agriculture.

In tropical conditions, some studies suggest that inoculation with non-symbiotic bacteria will not increase nitrogen fixation and plant yield. Alexander and Zuberer (1989) observed that inoculation with *Azospirillum lipoferum* had no effect on the amount of $^{15}N_2$ incorporated by maize, total nitrogen or plant dry matter. Döbereiner (1977) concluded that maize inoculation with *Azospirillum* in tropical soils offered little potential for increasing nitrogen fixation and plant yield because of the widespread distribution of *Azospirillum* in these soils. Baldani *et al*. (1986) concluded that the establishment of an inoculated microorganism depends on its

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competitiveness, that is influenced by plant cultivar, soil and climatic factors.

In India, *Azospirillum* inoculation increased yield in maize and sorghum (Tilak *et al*. 1982) and *Panicum miliaceum* (Rai 1988).

In temperate climates, inoculation with diazotrophic bacteria could be practical due to the rare occurrence of the microorganisms in soils of these regions. This is supported by the positive results obtained in a field study with wheat by Didonet *et al*. (1996) following inoculation with *A. brasilense* in the Brazilian South region.

In Israel, soils contain very low native populations of *Azospirillum* (Boddey and Döbereiner 1988). Under field conditions, *Azospirillum* inoculation in *Panicum miliaceum*, *Setaria italica*, sorghum and maize increased yield in different environmental and soil conditions with different fertiliser levels, in crops with and without irrigation (Kapulnik *et al*. 1981). In Egypt, inoculation with *Azospirillum* increased straw production and yield of maize (Hegazi *et al*. 1983) and wheat (Ishac *et al*. 1986).

The first promising results of maize inoculation with *Azospirillum* in Brazil were presented by Freitas *et al*. (1982). However, the authors warned against precipitate conclusions, indicating the need to confirm the findings in other soils and using different processes of diazotrophic inoculation.

Availability of energy and carbon sources plays an important role in the associative nitrogen fixation. The beneficial effect of soil organic matter on biological nitrogen fixation in grasses was studied by several authors, using different materials (Freitas *et al*. 1982; Hegazi *et al*. 1986; Ishac *et al*. 1986; Fallik and Okon 1988).

In view of the discordant data regarding the benefits of maize inoculation with *Azospirillum* and the shortage of research on soils of north-eastern Brazil, this study evaluated the benefits of maize inoculation in a Podzol Hydromorphic soil, representative of the Rain Forest Zone of Pernambuco, and the effect of addition of organic compounds on biological nitrogen fixation and maize yield.

Materials and methods

The experiment was arranged in a completely randomised factorial design with 3 replications (Pimentel Gomes 1985). The soil used was a Hydromorphic Podzol. Twenty samples of the surface layer (0–20 cm) were collected at random from the Itapirema Experimental Station in the Tropical Rainforest Zone of Pernambuco state (district of Goiana) in north-east Brazil. After collection, the soil samples were sieved (5 mm sieve), mixed and kept in clay pots to minimise the thermal shock resulting from excessive exposure to solar radiation. Analysis of soil, using the EMBRAPA (1997) methodology was: pH (H_2O) 4.6; P (Mehlich) 1mg/kg; exchangeable cations ($mmol_c$/kg)-Ca^{2+} + Mg^{2+} 15; K^+ 7.6; Al^{3+} 2; organic carbon 0.51%; total N 0.03%; sand 90%; silt 4%; clay 6%.

Four organic materials with different C:N ratios (solid vinasse, cow manure, vermicompost, and fruit-horticultural compost) (Table 1) were used. The solid vinasse is a byproduct of sugar cane processing; the vermicompost (earthworm cast) and the cow manure were obtained from the agricultural farm (UFRPE) at the Campus in Recife, Pernambuco; and the fruit-horticultural compost was produced by aerobic fermentation, using fruit-horticultural waste, from the Center of Agricultural Products (CEASA-Pernambuco). The organic materials were applied at levels normally applied by farmers, corresponding to 0, 10 and 20 t/ha. All organic materials were air-dried, sieved (5 mm sieve), well mixed and sampled (500 mg) to determine total N and organic carbon following the EMBRAPA (1997) methodology.

Table 1. Chemical characteristics of the organic materials used in the experiment.

Organic material	Total N	Total organic C	C:N ratio
	(%)	(%)	
Solid vinasse[1]	2.12	11.7	5.5:1
Cow manure	1.04	11.8	11.3:1
Vermicompost[2]	0.84	11.9	13.6:1
Fruit-horticultural compost[3]	0.35	7.2	20.6:1

[1] Byproduct of sugar cane processing.
[2] Earthworm cast.
[3] Product of aerobic fermentation of fruit-horticultural waste.

Seeds of maize (cv. CMS 22) were surface-sterilised with $HgCl_2$ (1%) for 5 minutes and 95% ethanol for 10 minutes, washed 6 times with sterilised water and soaked over night to imbibe water. Seeds were planted in trays containing peat:vermiculite (2:1 ratio); 10 days after sowing, seedlings were transplanted (5 seedlings/pot). Five days after seedling transplantation, plant numbers were reduced to 2 in each pot. The density of plants in each pot represented a plant population of 50 000 plants per hectare. Seven days after transplantation, each pot received a basic fertiliser application of phosphorus (0.66 g P) as $Ca(H_2PO_4)_2$ and potassium (0.50g K) as KCl, which is the recommended level for maize grown in Pernambuco state (IPA 1998). Magnesium and micronutrients were applied according to Norris (1964) using the micronutrient solution for tropical legumes.

In the treatments with *Azospirillum* inoculation, the strains NFB 2 and Sp 242 were applied individually. Strain NFB 2 was isolated from the rizosphere of maize grown in an acid soil of the UFRPE Campus. Strain Sp 242 was isolated from wheat and provided by the National Centre of Biological Resources in Rio de Janeiro (CNPAB — EMBRAPA). The bacterial culture consisted of the strains grown in a liquid medium free from nitrogen (NFb medium) and maintained at 30°C, for 48 hours (Döbereiner 1980). Inoculation with the bacterial culture occurred at seedling transplantation (1 ml/pot). A second inoculation occurred 10 days later (2 ml/pot). The treatments without inoculation received the same volume of the culture medium as described in the inoculated treatments.

Soil water was kept at 80% of field capacity, monitored by daily weighing. Plants were cropped 50 days after seedling transplantation (AST), and shoot dry matter was determined, as well as total N, using the auto-analyser Kjeltec (Model 1030), following the methodology described by Malavolta *et al.* (1989), and nitrogenase activity on washed out root systems (Boddey and Döbereiner 1982) by gas chromatography using the acetylene/ethylene technique (Hardy *et al.* 1973).

Enrichment cultures with superficially sterilised segments of roots were used in NFb medium (Döbereiner 1980). After 48 hours of growth at 30ºC, the occurrence of a white film in the middle surface of the medium culture indicated the growth of *Azospirillum* (Day and Döbereiner 1976), and the nitrogenase activity of the enrichment cultures (Moreira 1994) was determined by gas chromatography using the acetylene/ethylene technique (Hardy *et al.* 1973). Organic carbon and total N in soil were determined following the EMBRAPA (1997) methodology.

Results

Azospirillum *inoculation*

Inoculation with *Azospirillum* had no effect on dry matter production of maize but inoculation with strain NFB 2 in treatments receiving organic materials reduced total N accumulation by 24.6% relative to the control treatment (Figure 1).

Organic compounds application

The incorporation of organic materials generally increased both total N and dry matter accumulation in shoots. However, materials with narrower C:N ratios (cow manure and solid vinasse) produced progressive increases in both parameters at the higher level of application. The lowest responses occurred with fruit-horticultural compost. Plants that received this treatment incorporated 65% more nitrogen and 48% more dry matter than the control treatment without organic material (Table 2).

Table 2. Effect of the addition of organic material (OM) on shoot dry matter and total nitrogen.

O M level (t/ha)	Cow manure	Vermi-compost[1]	Fruit-horticultural compost [2]	Solid vinasse[3]	Means
			Total N in shoots (mg/pot)		
0	—	—	—	—	18.4c
10	60.9bA[4]	36.1aC	25.4aC	58.0bB	45.1b
20	150.1aA	39.4aC	35.2aC	120.7aA	86.4a
Means	105.5 A	37.8 B	30.3 B	89.4 A	
			Shoot dry matter (g/pot)		
0	—	—	—	—	4.6c
10	10.8bAB	8.9aBC	5.9aC	14.1bA	9.9b
20	23.3aA	8.4aB	7.8aB	23.6aA	15.8a
Means	17.0A	8.6 B	6.8 B	18.8 A	

CV (%) N total = 35.4 Shoot dry matter = 28.2

[1] Earthworm cast.
[2] Product of aerobic fermentation of fruit-horticultural waste.
[3] Byproduct of sugar cane processing.
[4] Within topics, values followed by different letters are significantly different (P = 0.05), using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns.

The addition of organic fertiliser to the soil significantly increased nitrogenase activity by microorganisms in maize roots as measured by acetylene/ethylene production (Table 3).

The nitrogenase activity in maize roots was affected by the type of organic fertiliser applied

(Table 3). Vermicompost produced the lowest level of activity and fruit-horticultural compost, the highest level.

Soil analyses after maize growth are presented in Table 4. Application of organic material affected both nitrogen and carbon content in soil, and the largest responses were obtained with cow manure and vinasse ($P < 0.05$). The increases in both soil N and soil C were greater ($P < 0.05$) with the higher level of organic fertiliser.

Table 3. Effect of the application of organic materials (OM) on nitrogenase activity in maize roots.

Treatments	Nitrogenase activity
Organic materials	(n moles C_2H_4 / h/ pot)
Fruit-horticultural compost[1]	922 a[2]
Cow manure	831 b
Solid vinasse[3]	804 b
Vermicompost[4]	685 c
O M levels (t/ha)	
0	603 c
10	728 b
20	893 a
CV (%) = 31.7	

[1] Product of aerobic fermentation of fruit-horticultural waste.

[2] Within topics, values followed by different letters are significantly different (P=0.05), using the Tukey test.

[3] Byproduct of sugar cane processing.

[4] Earthworm cast.

Table 4. Effect of application of organic materials (OM) on total N and organic C in soil after a maize crop.

O M level (t/ha)	Cow manure	Vermi-compost[1]	Fruit-horticultural compost [2]	Solid vinasse[3]	Means
			Total N in soil (%)		
0	—	—	—	—	0.037c
10	0.062bB[4]	0.056bBC	0.042aC	0.083bA	0.061b
20	0.097aB	0.080 aC	0.050aD	0.116aA	0.086a
Mean	0.079 B	0.068 C	0.046 D	0.099 A	
			Total organic C in soil (%)		
0	—	—	—	—	0.50c
10	1.01bA	0.96bA	0.80aB	0.96bA	0.93b
20	1.69aA	1.28aB	0.98aC	1.54aA	1.37a
Means	1.35 A	1.12 B	0.89 C	1.25AB	

CV (%) Total N in soil = 16.2 Total organic C in soil = 18.5

[1] Earthworm cast.

[2] Product of aerobic fermentation of fruit-horticultural waste.

[3] Byproduct of sugar cane processing.

[4] Within topics, values followed by different letters are significantly different (P=0.05), using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns.

Discussion

The failure of *Azospirillum* inoculation to affect dry matter production in maize supports the findings of Tilak *et al.* (1982), Boddey *et al.* (1986) and Alexander and Zuberer (1989), but is at variance with results obtained by Ishac *et al.* (1986) and Rai (1988). The absence of a response may be a function of the presence of native *Azospirillum* species and/or other micro-aerobic nitrogen-fixing organisms. Their presence was confirmed by bacterial growth and high nitrogenase activity in the enrichment cultures of all treatments (data not presented) with no significant difference between treatments.

The tendency for *Azospirillum* inoculation to reduce N accumulation in the presence of applied organic materials is in agreement with the reduction in total N in maize grain observed by Freitas *et al.* (1982) and in wheat by Baldani *et al.* (1983). Boddey *et al.* (1986) suggested that *Azospirillum* inoculation frequently decreased plant nitrogen content but we did not observe this effect when organic fertilisers were not applied.

Magalhães *et al.* (1979) suggested that *Azospirillum* inoculation using denitrificant strains could decrease total N in plants. On the other hand, Christiansen-Weniger *et al.* (1985) affirmed that *Azospirillum* could influence the assimilation of nitrate. It is well known that endophytic diazotrophic bacteria produce substances that can stimulate plant growth and could induce morphologic modifications in roots (Bashan 1998; Baldani *et al.* 1999). These effects could vary between species and strains of bacteria (Goi *et al.* 1998), and sometimes between plant isolines (Garcia de Salomone *et al.* 1996).

The increase of maize growth in response to organic matter addition is consistent with the conclusion of Hegazi *et al.* (1983) who obtained responses to the application of maize straw, a material with high C:N ratio, in the nitrogenase activity on the roots of maize. They suggested that the straw acted as a source of carbon and energy and intensified nitrogen fixation by removing mineral nitrogen from the soil through N immobilisation by soil microbes. On the other hand, intense liberation of mineral N in organic materials with high N concentration and low C:N ratios could decrease nitrogen fixation by an inhibitory effect of mineral N in the growth of microorganisms involved in the process (Baldani *et al.* 1986; Ishac *et al.* 1986; Minhoni *et al.*

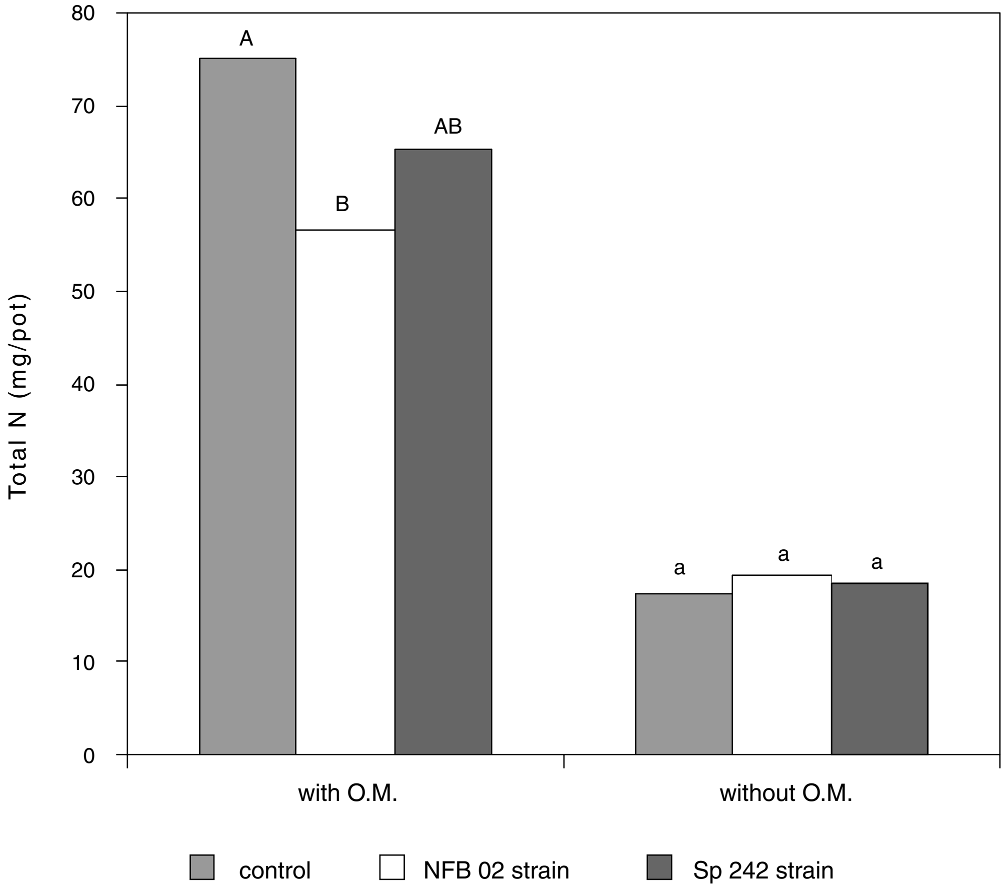

Figure 1. Total N accumulated in shoots of maize inoculated with *Azospirillum*, with and without organic material addition. Different letters on top of columns indicate significant differences (P=0.05), using the Tukey test. Upper case letters compare data with organic material addition and lower case letters compare data without organic material addition.

1990). Numerous reports (Fallik and Okon 1988; Berton *et al*. 1989; Ros *et al*. 1993) have shown the benefits of organic matter application to maize crops, as well as the importance of the C:N ratio of the materials.

We conclude that application of organic compounds to soil will increase dry matter production and total N accumulation in maize, especially when applied as cow manure and solid vinasse. This was associated with an increase in associative nitrogenase activity on the maize roots. *Azospirillum* inoculation was not effective in nitrogen fixation and growth of maize in Brazilian rainforest soil. The negative effect of *Azospirillum* inoculation on total N accumulation in rainforest soil underscores the need for further research to study in detail the influence of inoculation with associative bacteria under field conditions, using different soils and plants.

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