Response of maize (*Zea mays* L.) to straw amendment and inoculation with N_2-fixing bacteria in a tropical soil.

F.B. Mwaura
Dept. of Botany, Univerity of Nairobi, Chiromo,
P.O. Box 30197,
NAIROBI.
Kenya.

SUMMARY:

Acetylene reduction activity (ARA) was low in unamended soil (trace - 0.84 n mol C_2H_4/tube/h). Large ARA increases were observed after soil amendment with organic carbon sources. A straw - soil system had a mean ARA of 1.5 μ mol C_2H_4/tube/h while the mean ARA in a straw - soil - plant system was 2.2 μ mol C_2H_4/tube/h after two weeks. Inoculating the maize plants with diazotrophic rhizosphere bacteria raised the shoot dry weight by up to 15.4% in three-week-old plants under greenhouse conditions. Inoculation also increased shoot lengths and root biomass by 8.8 and 20% respectively. Incorporation of straw in the soil raised the root biomass by 36-63% and decreased the shoot/root ratio from 1.47 to 0.94.

INTRODUCTION

Non-symbiotic nitrogen fixation and its ecological significance has been cited as one of the most controversial problems in soil biology (Dommergues et al., 1973). The scarcity of available carbon and energy substrates is known to be a major factor limiting non-symbiotic nitrogen fixation in soils and grass-bacteria associations (Spiff and Odu, 1972; Abd-El-Malek, 1971; Okafor 1977; Hegazi, 1983). Besides increasing heterotrophic nitrogen fixation, straw incorporation after crop harvests, as an agricultural practice, returns various nutrients to the soil. Lynch et al. (1984) have also suggested that amendment of soil with straw and its subsequent decomposition may lead to the production of soil stabilizing agents which may help to minimise soil loss in erosion prone areas.

Despite the potential benefits accrued from straw incorporation in the soil, straw is frequently burned in the tropics and elsewhere. It is only in China and Vietnam where soil amendment with straw is a common practice (Watanabe, 1984). In Kenya, straw is normally removed by burning. The following is a preliminary investigation into the potential use of straw as a substrate for cooperative N_2 fixation in a soil-plant system under controlled conditions.

MATERIALS AND METHODS

Effect of Carbon Substrate Amendment on Soil ARA

The organic substrates were glucose, sodium malate and macerated dried maize stalks. Approximately 10g of the soil was put in each of several 30ml. bottles. The substrates were added (5% W : W) and mixed well with the soil. Five replicate soil samples were set up for each of the carbon substrates. The amended soils were then moistened well with water and the bottles were sealed tightly with rubber closures. Bottles containing unamended soils were similarly prepared. All soils samples were aerobically incubated under 10% acetylene at 24°C. Control samples were set up without acetylene addition. Gas samples were removed and analysed for ethylene content in a gas chromatograph over a 72 h period.

Response of Maize seedlings to straw amendment and inoculation

Soil Preparation.

Washed and sterile vermiculite was put in large test tubes (30mmx320mm) to a depth of 2-3cm. The vermiculite was moistened well with sterile water and then covered with a layer of moist soil amended with macerated maize stalks (5% W:W) from a harvested crop. The soil depth was 3-4 cm. A second batch of test tubes was similarly prepared using vermiculite and unamended soil.

Inoculant preparation

Two vigorous acetylene-reducing bacteria strains J and L had previously been isolated from roots of maize plants grown in the experimental soil (Mwaura, unpublished data). Both strains were motile Gram-Ve rods whose identities have yet to be confirmed. The bacteria were grown in a liquid nitrogen-deficient NFb-glucose medium (Lindberg and Granhall, 1984) as stagnant cultures for four days at 28°C. The cultures were centrifuged (Wifug Centrifuges, Bradford, England) and washed twice with sterile 0.05M phosphate buffer pH 7.0. The cells were finally resuspended in the buffer to give an optical density of 0.8 units at 560mm. Optical density measurements were made in a Shimadzu Spectrophotometer UV - 120 - 01.

Seed Preparation, Inoculation and Plant Growth

Maize seeds (*Zea mays* L) cultivar LG11 were washed and surface sterilized in acidified mercuric chloride (Vincent, 1970). The seeds were thoroughly washed in several changes of sterile water and then germinated aseptically on water agar plates in the dark. Seedlings of uniform size were selected after two days. One seedling was sown in each of the prepared test tubes. Some of the seedling in both the unamended and straw amended soil were inoculated with 0.2 ml.bacteria suspension of strain J. The other seedlings were similarly inoculated with stain L. Uninoculated seedlings were drenched with 0.2 ml. of the sterile buffer. In each treatment six replicates were prepared. All seedlings were covered with a little soil and finally a thin layer of vermiculite was applied to exclude light. The test tubes were finally sealed with parafilm (Scher *et al.*, 1984) to minimise contamination and water loss. All tubes were then placed in a greenhouse and illuminated with 400W greenhouse lamps (phillips, Holland). The temperature was regulated at $25^{o}\pm2^{o}C$ and a light/dark cycle of 16/8 h respectively was maintained. The experiment was terminated after three weeks when the plants attained their maximum size possible in the tubes. The plant shoot lengths were measured and the plants were harvested and dried to constant weight at $80^{o}C$ in a forced air oven.

Acetylene reduction assays of the soil-plant system

At weekly intervals the parafilm seals were replaced with gas tight rubber bungs modified to carry suba seal caps. The plants were incubated aerobically under 10% acetylene for 4 h. No acetylene was added to control test tubes within the treatments. Gas samples were withdrawn and analysed for ethylene in a gas chromatograph (GC 428 Packard Instruments, The Netherlands). After each assay the rubber bungs were removed and the test tubes were flushed with air before resealing them with parafilm.

Effect of Straw amendment on soil ARA over a prolonged period

The experimental soil was mixed with macerated maize stalks (5% W:W) and 10g samples were transferred into large test tubes (25mmx300mm) containing a little washed and sterile vermiculite moistened with water. Other test tubes were similarly prepared with the unamended soil. Five replicate testtubes were set up with each soil. All soil samples were moistened with sterile water and a thin layer of vermiculite was applied to minimise growth of cyanobacteria. The testtubes were then sealed with parafilm and held upright in dark cardboard boxes in the greenhouse. The ARA was measured weekly for six weeks as described earlier on for the plant-soil system. Endogenous production of ethylene was always checked for in soil samples incubated without acetylene.

RESULTS

Effect of Substrates on Soil ARA

Soil amendment with glucose and the plant residues greatly stimulated the ARA (Table 1). Addition of the maize straw, macerated pith from dried maize stalks, enhanced soil ARA by about thirty times within the first 24 h. Continued incubation of the soil samples for 72 h showed an increase in ARA by nearly three thousand times over that of the unamended soil.

Table 1. Effect of soil Amendment with carbon substrates on the ARA

	n mol C_2H_4/bottle*		
Substrate	24h	48h	72h
Glucose	12.9	1517.3	8568
Malate	7.4	7.8	11.0
Plantresidue	189	7500.2	22539.8
Control	6.6	7.4	7.9

* Each bottle contained about 8g dry weight soil.

Soil samples in 30 ml McCartney bottles were amended with 5% (W:W) glucose, sodium malate or macerated maize straw. Unamended samples were used as controls. All soil samples were moistened with water and aerobically incubated under 10% acetylene at 28°C. Results are means of four replicates.

Glucose stimulated the soil ARA but to a lesser extent than the plant residue (Table 1). Amendment of the soil with malate had little or no effect on the ARA even after prolonged incubation under acetylene.

Crop response to straw amendment and inoculation

Plant Growth

In the unamended soil, bacteria strains J and L raised the shoot dry matter yield of the inoculated maize plants by 12.8 and 15.4% respectively over the uninoculated plants (Table 2). Inoculation with strains J and L also increased root biomass by 15 and 20% respectively. Whereas inoculation with strain J increased shoot lengths by 8.8%, over the uninoculated plants, strain L had little or no effect.

Table 2. Response of maize plants to straw amendment and inoculation with diazotrophic rhizosphere bacteria.

	Inoculant	Shoot Length (cm)	Shoot d.w. (mg)	Root d.w. (mg)	S/R Ratio
Unamended Soil	Control	23.8±0.8	142.4±6.3	9.9±3.3	1.470
	J	25.9±1.9	160.6±12.3	111.5±9.6	1.440
	L	24.2±1.6	164.4±17.8	116.3±8.8	1.413
Soil + Straw	Control	18.7±0.6	148.5±8.5	158.3±14.2	0.938
	J	19.9±0.6	141.3±11.5	151.3±15.7	0.934
	L	19.5±0.6	154.0±7.6	160.1±7.1	0.962

Maize seedlings cultivar LG 11 were inoculated with bacteria strains J and L and grown in unamended and straw amended soils for three weeks in a greenhouse. Plants were harvested and their shoot lengths, shoot and root dry weights determined. Results are means of six replicates ± S.E.

Soil amendment with straw (5% W:W) lowered the shoot dry matter yield relative to the total plant dry weight during the experimental period (Table 2). Shoot development was clearly inhibited from the second to the tenth or so day after the maize seedlings were transfered to the straw incorporated soil. During this period, the maize plant roots remained close to the soil surface. After ten or so days, normal downward growth of the roots through the straw incorporated soil was observed. Inoculation with bacteria strains J and L did not increase the plant dry weight. In comparison with the unamended soils, straw incorporation raised the root biomass by about 36% in inoculated and 63% in uninoculated plants. These large increases in root biomass in the straw amended soil were also reflected in the low S/R ratios (Table 2).

Acetylene Reduction Activity in Intact Plants

The ARA detected in the soil-plant systems was highly variable in all treatments. In unamended soil, seedling inoculation with the acetylene reducing bacteria did not raise the number of nitrogenase positive maize plants compared to the control plants. On the whole, inoculation with bacteria strains J and L failed to increase plant associated ARA. A lower nitrogenase activity was recorded among the inoculated than the uninoculated plants (Table 3).

Table 3. ARA associated with inoculated maize plants in a straw amended and unamended soil over a three- week period.

		n mol C_2H_4/tube*/4h					
		Week 1		Week 2		Week 3	
	Inculant	Mean ARA	Range	Mean ARA	Range	Mean ARA	Range
Soil	Control	74.3	0-264	115.2	13.2-211.2	852.5	72.6-3124
	J	20.7	0-92.4	31.5	0-151.8	20.9	0-71.5
	L	8.8	0-33	133.3	16.5-495	17.6	trace-59.4
Soil + Straw	Control	429	110-1293.6	8595.9	616-34215	666.4	264-1540
	J	123.2	13.2-275	187	33-324.5	541.4	5.6-1298
	L	121.4	22-356.4	221.5	27.5-534.6	102.3	33-242

* One maize plant was sown per test tube.

Maize seedlings cultivar LG 11 were inoculated with bacteria strains J and L and grown in straw amended and unamended soils for three weeks in a greenhouse. The plants were assayed weekly for ARA. Results show mean and range of five replicates. No ethylene was detected in tubes incubated without acetylene.

Very high rates of ARA were observed in soil - plant systems where straw had been added (Table 3). Detectable nitrogenase activity was evident in all replicates in both inoculated and uninoculated treatments.

In most of the replicates, ARA was highest during the second week when activities of up to 8.6 u mol. C_2H_4/tube/h were recorded. The nitrogenase activity however varied considerably and inoculation did not increase plant-associated ARA during the experimental period.

Straw amended Soil ARA over a prolonged period

Nitrogenase activity increased tremendously (trace 1.5 μ mol C_2H_4/tube/h) in the straw incorporated soil over the first two weeks. After this period the activity dropped and at six weeks was just about a third of that recorded at two weeks (Fig. 1). In the unamended soil, a very low acetylene reduction activity (trace - 0.84 n mol C_2H_4/tube/h) was detected throughout the experimental period.

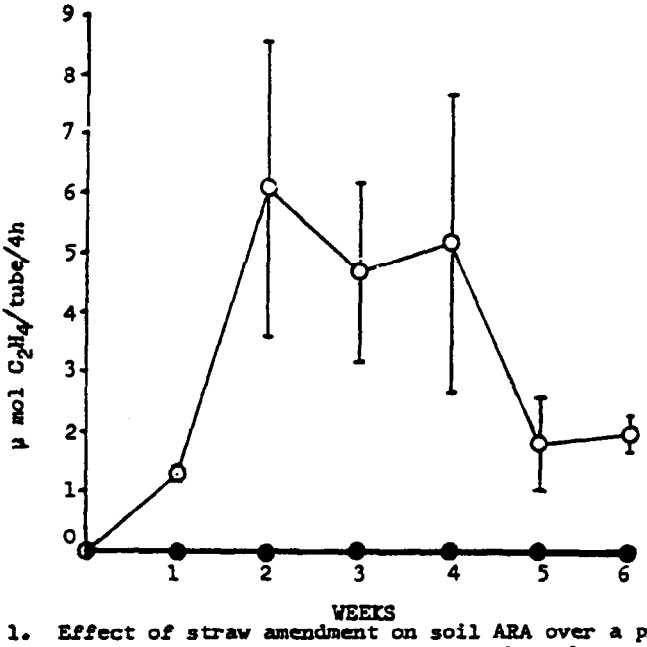

Fig. 1. Effect of straw amendment on soil ARA over a prolonged period. Unamended ●——● and straw amended soils O——O were assayed weekly for ARA. Results are means of five replicates. Bars represent standard error.

DISCUSSION

The results obtained in this study indicate that heterotrophic nitrogen fixation in this tropical soil is energy limited. However malate was unsuitable as a substrate while glucose and the plant residue greatly stimulated nitrogenase activity in the soil. Reports indicate that glucose and other sugars increase rhizosphere soil ARA most effectively while malate is most effective in increasing ARA of excised roots (Boyle and Patriquin, 1981). It is possible that certain factors present in the plant material may have stimulated growth of the diazotrophs considerably which resulted in such high levels of ARA.

In the greenhouse experiment, maize plants clearly induced nitrogenase activity in indigenous N_2 fixing bacteria present in the soil. The soil-plant system had ARA of up to 781 n mol C_2H_4/tube/h while soil alone had a

negligible 0.84 n mol C_2H_4/tube/h. There was little or no ARA attributable to cyanobacteria present in the soil. The high mean ARA recorded in the straw-soil-plant system (2.2 μ mol C_2H_4/tube/h) may have been largely due to heterotrophic N_2-fixing bacteria present in the soil. This is suspected since this high ARA detected in the second week, was in the same range with that recorded in the straw-soil plant free system (1.5 μ mol C_2H_4/tube/h) within the same period (Fig. 1). The difference in ARA (0.7 μ mol C_2H_4/tube/h) between the two systems is within the range of ARA detected in the unamended soil-plant system (up to 781 n mol C_2H_4/tube/h).

Inoculation of the maize plants with the two N_2-fixing bacteria strains J and L failed to increase plant associated ARA. It is possible that the two strains could not successfully compete with the indigenous microflora. A suggestion has been made that antagonistic processes tend to eliminate the introduced bacteria strain which may result in failure of inoculation to increase nitrogen fixation (Diem and Dommergues, 1980). On the other hand the numbers of these two bacteria strains, previously isolated from maize plants grown in the same soil, may have been high in the soil such that inoculation effects in terms of plant-associated ARA were minimised.

Increases in crop yields after inoculation with bacteria have been reported for wheat (Rai and Gaur, 1982; Mertens and Hess, 1984; Millet and Feldman, 1984; Reynders and Vlassak, 1982), forage grasses (Bouton and Zuberer, 1979; Smith et al. 1978), barley (Fayez and Vlassak, 1984; Tilak and Murthy, 1983) rice and others (subba Rao, 1980 and 1981). The increases of up to 15.4% in dry weight of maize plants, observed in this study due to inoculation may be significant bearing in mind that the plants were grown for only three weeks under suboptimal conditions. Hegazi et al. (1983) reported 50% increases in the dry weight of twelve-week-old maize plants inoculated with Azosphirillum under greenhouse conditions. Similar increases in the dry weight of inoculated maize plants have also been claimed (Cohen et al. 1980; O'Hara et al. 1981; Nur et al. 1980). It is possible that the observed increases in plant dry weight after inoculation may have been largely due to other reasons rather than increased rates of nitrogen fixation. Phytohormonal influence (Tien et al., 1979, Brown, 1972) and enhancement of mineral uptake by plant roots (Lin et al., 1983) have been cited as possible explanationsfor increases in the dry weight of inoculated plants. However Suslow (1982) suggested that plant weight increases observed after inoculation may result from inhibition and alteration of the normal root microflora by the inoculant strains.

From these preliminary studies it would appear that straw incorporation in this tropical soil may rapidly enhance its nitrogen status when adequate moisture is available. Further investigations will be carried out in this direction. The large increases in root biomass in a straw amended soil may be significant in the later growth and development of the plants. The initial inhibitory effects of straw amended soil on seedling establishment may possibly be avoided by sowing the crop a few weeks after the straw has been incorporated into the soil.

Acknowledgement:

I wish to acknowledge financial support from The Swedish Institute in Stockholm, during the study. I am also indebted to Prof. U. Granhall for material support.

REFERENCES

Abd-El-Malek, Y (1971). Free-living nitrogen-fixing bacteria in Egyptian soils and their possible contribution to soil fertility. Plant Soil, Special Volume, 423-442

Bouton, J.H. and Zuberer, D.A. (1979). Response of Panicum maximum Jacq to inoculation with Azospirillum brasilense. Plant Soil 52, 585-590

Boyle, C.D. and Patriquin, D.G. (1981) Carbon metabolism of Spartina Alterniflora Loisel. in relation to that of associated N_2 fixing bacteria. New Phytologist 89, 275-288.

Brown, M.E. (1972). Plant Growth substances produced by microorganisms of soil and rhizosphere. J. appl. Bact. 35, 443-451.

Cohen, E., Okon, Y, Kigel, J., Nur, I. and Henis, Y (1980). Increase in dry weight and total nitrogen content in Zea mays and Setaria italica associated with Nitrogen-fixing Azosphirillum spp. Pl. Physiol. 66, 746-749.

Diem, H.G. and Dommergues, Y.R. (1980). Significance and improvement of rhizospheric N_2 fixation. In Recent Advances in Biological Nitrogen Fixation (Subba Rao, N.S. ed.) 190-226. Edward Arnold; London.

Dommergues, Y. Balandreau, J., Rinaudo, G. and Weinhard, P. (1973). Non-symbiotic nitrogen fixation in the rhizospheres of rice, maize and different tropical grasses. Soil Biol. Biochem. 5, 83-89.

Fayez, M. and Vlassak, V. (1984). Effect of inoculation with Azospirillum brasilense on barley grown under semi-arid conditions. Zbl. Mikrobiol. 139, 359-365.

Lin, W., Okon, Y., and Hardy, R.W.F. (1983). Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillum brasilense. Appl. Environ. Microbiol. 45, 1775-1779.

Hegazi, N.A., Monib, M., Amer, H.A. and Shokr E. (1983). Response of maize plants to inoculation with azospirilla and (or) straw amendment in Egypt Can. J. Microbiol. 29, 888-894.

Lindberg, T. and Granhall, U. (1984), Isolation and characterization of Dinitrogen-Fixing bacteria from the Rhizosphere of Temperate cereals and forage grasses. Appl. Environ. Microbiol. 48,683-689.

Lynch, J.M. Harper, S.H.T., Chapman, S.J. and Veal, D.A. (1984). Approaches to the controlled production of novel agricultural composts. In Methods for Environmental Biotechnology (Grainger, J.M. and Lynch,J.M. eds.) 69-78. Academic Press Inc. : London.

Mertens, T. and Hess, D. (1984). Yield increases in spring wheat (Triticum aestivum L.) inoculated with Azospirillum lipoferum under greenhouse and field conditions of a temperate region. Plant Soil 82, 87-99.

Millet, E. and Feldman, M. (1984). Yield response of a common spring wheat cultivar to inoculation with Azospirillum brasilense at various levels of nitrogen fertilization. Plant Soil 80, 225-259.

Okafor, N. (1977). Non-symbiotic nitrogen fixation in tropical humid soils with particular reference to Nigeria. In Biological Nitrogen Fixation in Farming Systems of the Tropics. (Ayanaba, A. and Dart, P.J. eds.) 267-272.

O'Hara, G.W., Davey, M.R. and Lucas, J.A. (1981). Effect of inoculation of Zea mays with Azospirillum brasilense strains under temperate conditions. Can. J. Microbiol. 27, 871-877

Rai, S.N. and Gaur, A.C. (1982). Nitrogen fixation by Azospirillum spp. and effect of Azospirillum lipoferum on the yield and N-uptake of wheat crop. Plant Soil 69, 233-238.

Reynders, L. and Vlassak, K. (1982). Use of Azospirillum brasilense as biofertilizer in intensive wheat cropping. Plant Soil 66, 217-223.

Scher, F.M., Ziegle, J.S. and Kloepper, J.W. (1984). A method for assessing the root-colonizing capacity of bacteria on maize. Can J. Microbiol. 30, 151-157.

Smith, R.L., Schank, S.C., Bouton, J.H. and Quesenberry, K.H. (1978). Yield increases of tropical grasses after inoculation with Spirillum Lipoferum. In Environmental Role of Nitrogen-fixing Blue-green algae and Asymbiotic Bacteria (Granhall, U. ed.) Eco. Bull. (Stockholm) 26,380-385.

Spiff, E.D. and Odu, C.T.I. (1972). An assessment of non-symbiotic nitrogen fixation in some Nigerian soils by the acetylene reduction technique. Soil Biol. Biochem. 4, 71-77

Suslow, T.V. (1982). Role of root-colonizing bacteria in plant growth. In Phytopathogenic prokaryotes (Mount, M.S. and Lacy, G.H. eds.) 1,187-223. Academic Press : New York.

Subba Rao, N.S. (1980). Crop response to microbial Inoculation. In Recent Advances in Biological Nitrogen Fixation (Subba Rao, N.S. ed.) 406-420.

Subba Rao, N.S. (1981). Response of crops to Azospirillum inoculation in India. In Associative N_2-fixation Vol.1 (Vose, P.B. and Ruschel, A.P. eds.) 138-144. CRC Press Inc. Florida, U.S.A.

Tien, T.M. Gaskins, M.H. and Hubbel, D.H. (1979). Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetum americanum L.) Appl. Environ. Microbiol. 37, 1016-1024.

Tilak, K.V.B.R. and Murthy, B.N. (1983). Response of barley (Hordeum Vulgare) to inoculation with Azospirillum brasilense. Current Science 52, 257-258.

Vincent, J.M. (1970). A manual for the Practical Study of Root-module Bacteria. Blackwell Scientific Publications : Oxford.