RELATIONSHIP BETWEEN SOIL NITROGEN AND FREE NITROGEN FIXER POPULATION UNDER SOME LEGUME TREES IN ARID AND SEMIARID AREA

Vrinda Thaker[1] - Y.D. Singh[1]

ABSTRACT

The free nitrogen fixer population and soil total N was studied under the four tree species viz. A. nilotica, P. juliflora, L. leucocephala and D. sissoo, in arid to semiarid area of Saurashtra (Rajkot). The samples were collected from the rhizosphear of the tree species. The total bacterial count and soil C were also studied under all four tree species. The relationship between free N fixer and total soil N is discussed.

Keywords: Rhizosphere legume trees, free-living bacteria, Azotobacter, Acacia, Leucaena, Prosopis, Dalbergia.

COMPENDIO

RELACION ENTRE EL NITROGENO DEL SUELO Y LA POBLACION DE FIJADORES DE NITROGERNO DE VIDA LIBRE EN LA RIZOSFERA DE CUATRO LEGUMINOSAS ARBOREAS

En las áreas áridas y semi-áridas de Saurashtra (Rajkot, India), se colectaron muestras de suelo en la rizosfera (5, 15 y 25 cm) de plantaciones de 2 edades de cuatro leguminosas arbóreas. El máximo N libre se registró en Acacia nilotica a 5 cm de profundidad, el cual disminuyó a los 25 cm en 25 y 75% en las plantaciones de menor (17 meses) y mayor edad (29 meses) respectivamente. El contenido de C sólo se incrementó en A. nilotica en 25% a 5 y 15 cm. Los conteos mas altos de Azotobacter se registraron a los 29 meses en Dalbergia sissoo y a los 17 meses en Prosopis juliflora; los niveles mínimos ocurrieron en Leucaena leucocephala en las dos edades. (HQV).

Palabras clave: Rizosfera de leguminosas arbóreas, Bacterias de vida libre, Azotobacter, Acacia, Leucaena, Prosopis, Dalbergia.

INTRODUCTION

The nutrient reserve of a forest is held in the foliage, bark, branches and sap wood of the trees, in the soil and in the understorey vegetation. A steep rise in fertilizer prices and concern for conservation of non-renewable energy resources has forced scientists, especially of developing countries, to emphasize burial of legume crops in order to save energy-intensive nitrogenous fertilizers and enhance crop yield (Turvey and Smethurst, 1983). Further, human need for fuelwood, food, fodder, erosion control and soil improvement in arid and semi-arid regions has led to an increased interest in tree legumes and their use in agroforestry systems (Felker, 1979; Oldeman, 1983). There is a wide range of nitrogen fixing plants that have been used in forestry with the presumed objective of raising soil nitrogen levels and subsequently improving the growth of the non nitrogen fixing forest species.

Apart from the objective of fixing nitrogen, there have been few objectives common to the management of nitrogen fixing plants in managed forests. In some cases the nitrogen fixing plant may have an intrinsic economic value as timber or fodder, and hence be managed and harvested as intensively as the forest crop either through rational cropping or as plant mixture. In other instances, the nitrogen fixing plants may have no value other than for their ability to fix nitrogen and hence may be ploughed into the soil or left as a mixture with the forest to fix nitrogen through forest rotation (Turvey and Smethurst, 1983). On the other hand, the ecological significance of non-symbiotic ni-

[1] Department of Biosciences, Saurashtra University. Rajkot - 360005, India.

trogen fixation in the rhizosphere is one of the most controversial problem in the field of soil biology. Some authors who have studied the role of *Azotobacter* in the rhizosphere, think that the contribution of this bacterium to nitrogen enrichment is negligible (Mishutin and Shilnikova, 1969). Additionally, it has been suggesated that, at least under tropical climates and in the rhizosphere of some plants, non-symbiotic nitrogen fixation must not be overlooked (Dobereiner, 1968). Therefore, in the present study, free nitrogen fixers population was determined under the various tree species in relation to soil nitrogen.

MATERIALS AND METHODS

Soil nitrogen and carbon content

For the estimation of total N the Kjeldahl procedure was employed. The soil samples were taken from various depths i.e. 5 cm, 15 cm and 25 cm from the ground, near the trees. The samples were taken from all the tree species (*A. nilotica, P. juliflora, L. leucocephala* and *D. sisoo*) studied, from 29 months and 17 months old plantation and these sites were designed as Site I and Site II plantation respectively. The control samples were collected from an open area very near to the plantation sites. The soil samples were oven dried for 48 h and sieved with 0.5 mm sieve. In Kjeldahl digestion flasks, 1 g of soil sample was mixed with 5 ml of water and digested with 30 ml of sulphuric acid. Since K_2SO_4 and $CuSO_4$ are known to function as catalyst in increasing the rate and temperature of digestion, they were added (0.5 g and 1.0 g respectively) with suplhuric acid. The digestion flask was kept on heater at 200°C temperature for 3 h. It was cooled to room temperature and final volume was made up to 100 ml with DW. The digest was shaken properly and 5 ml was taken into Kjealdahl assemble.

For the determination of ammonia, N liberated by digestion process was collected in Erlenmeyer flask containing 25 ml 4% H_3BO_3 and few drops of mixed indicator solution (0.5 g Bromocresol green and 1 g methyl red in 100 ml 95% ethyl alcohol) and placed under the condenser of the assembly and titrated with N/14 H_2SO_4. Total N was calculated following the formula:

$$\% N = \frac{N (T - B) 1.4}{S}$$

donde,

N = Normality of acid
T = Sample titration reading of standard acid
B = Blank titration readin
S = Soil weight in g

For the estimation of % C, 500 mg soil sample was taken in 500 ml Erlenmeyer flask and 10 ml $K_2Cr_2O_7$ (1M) and 20 ml of concentrated H_2SO_4 was added and kept on asbestos sheet for 30 minutes. After cooling it to room temperature, 100 ml of DW was added. Prior to titration with ferrous ammonium sulphate (1M), 3-4 drops of ferrin indicator was added. The end point was determined by the change in colour from green to blood red. The blank determination was made following the procedure described above, except soil sample.

All the estimations were done in triplicate from all the four species, from both the sites.

Total bacterial count and free nitrogen fixer

The viable plante count method is one of the most common procedure for the enumeration of bacteria. In this procedure serial dilutions of a suspension of bacteria are plated on to a suitable solid growth media. The media consisted of g/l of following constituents:

K_2HPO_4 0.1; $MgSO_4$ 0.2; $CaCl_2$ 0.1; NaCl 0.1; $FeCl_3$ 0.002; KNO_3 0.8, Asparagine 0.5 and mannitol 0.1 and the pH of the medium adjusted to 7.0, before autoclaving.

From the rhizosphere of the plant species, soil samples were collected and 1 g of the soil sample was suspended in 10 ml DW. Serial dilutions were prepared ranging from 10^{-1} to 10^{-8} with sterile DW. From 10^{-6}, 10^{-7} and 10^{-8} times diluted suspensions, 0.1 ml solution was spread over the solid growth medium and incubated for 96 h. Number of bacterial colonies formed were counted.

Azotobacter

To find out population of *Azotobacter* from the soil sample, serial dilutions of bacterial suspension (0.1 ml) were spread over the solid growth medium. The sample was incubated with medium containing sucrose 20 g, K_2HPO_4 0.8 g, KH_2PO_4 0.2 g. Na_2MoO_4 0.1 mg, $MgSO_4.7H_2O$ 0.41 g, $CaCl_2.2H_2O$ 0.1 g, $Na_2FeEDTA$ 0.1 mg in final volume one litre and pH adjusted to 7.0. Colonies of free nitrogen fixers were counted after 96 h for all the four tree species.

RESULTS AND DISCUSSION

Changes in total % N at different soil depths are presented in *Table Ia*. In *A. nilotica*, maximum free N was recorded in the upper 5 cm depth of the soil in

Table 1. Levels of % N and % C in the soil samples of different tree species collected from different depths from two sites. AN *A. nilotica*, PJ- *P. juliflora*, LL - *L. leucocephala*, DS- *D. sissoo*.

Table 1a: PERCENT N

SPECIES	SITE	5 cm	15 cm	25 cm
AN	I	0,18	0,085	0,06
	II	0,15	0,07	0,02
PJ	I	0,16	0,12	0,09
	II	0,15	0,13	0,09
LL	I	0,24	0,18	0,16
	II	0,15	0,30	0,18
DS	I	0,20	0,20	0,17
	II	0,20	0,20	0,14
Control soil		0,06	0,06	0,08

Table 1b: PERCENT C

SPECIES	SITE	5 cm	15 cm	25 cm
AN	I	5,05	5,09	4,51
	II	4,12	2,94	2,40
PJ	I	4,32	4,24	3,86
	II	4,83	5,03	4,44
LL	I	3,74	6,67	3,17
	II	3,69	3,22	3,35
DS	I	4,79	4,18	3,90
	II	4,46	4,50	3,56
Control soil		4,25	4,08	4,16

both 17 months (site II) and 29 months (site I) old trees. At 25 cm depth, the levels decreased to 25% and 75% in smaller and larger trees, respectively. In case of *P. juliflora*, in upper 5 cm depth, nearly 250% increase in % N was recorded while in 15 cm and 25 cm depths the increase was 200% and 113% respectively. In *L. leucocephala*, maximum increase in N levels was recorded in comparison to all the tree species studied. This increase was nearly 400% at 5 cm depth in larger trees while in smaller trees at 15 cm depth it was 500%. Even at 25 cm depth more than 200% N was recorded in both the trees. In *Dalbergia sissoo* both at 5 cm and 15 cm depths, nearly 330% increase in N level was recorded in the smaller as well as in the larger trees. However, percent C content showed nearly 25% increase in *A. nilotica*, at 5 cm and 15 cm depth. In other species, the percent C content was lower than the control value (*Table 1b*).

Total bacterial counts and *Azotobacter* counts from soils of smaller and larger trees are reported in *Table 2*. Total bacterial counts were higher in soil samples collected from smaller and larger plantations of *D. sissoo*. In larger trees, free nitrogen fixer-Azotobacters higher counts in *D. sissoo*, followed by *A. nilotica*, *P. juliflora* and minimum counts were recorded in *L. leucocephala*. In smaller trees, the level of *Azotobacter* was highest in *P. juliflora* followed by *A. nilotica*, *D. sissoo* and minimum levels in *L. leucocephala*. In comparison with larger trees, the smaller trees recorded very high amount of free fixer in the soil, except in *L. leucocephala*, where the levels remained almost same. It has been reported that small leucaena seedlings develop a substantial taproot system and have nitrogen fixing rhizobium nodules (Pcarr, 1981). Since leucaena is a fast growing species, amongst the species studied, low count of free nitrogen fixers may be due to the capability of leucaena to fix nitrogen symbio-

Table 2. Total bacterial counts and *Azotobacter* counts from the soil sample of larger (A) and smaller (B) trees. Other details as per Table 1.

SPECIES	TOTAL COUNT	AZOTOBACTER
A		
AN	32×16^6	18×10^2
PJ	04×10^6	13×10^2
LL	03×10^6	12×10^2
DS	90×10^6	22×10^2
B		
AN	10×10^5	44×10^3
PJ	60×10^6	22×10^4
LL	28×10^7	11×10^2
DS	109×10^8	32×10^2
Control soil	06×10^6	02×10^1

tically. It has been reported that leucaena fix more than 500 kg nitrogen/ha (Pcarr, 1981). Higher levels of nitrogen in the soil under the leucaena plantation (*Table 1*), further support this conclusion.

The occurrence of *Azotobacter* in soil is influenced by a number of factors, such as pH (Jensen, 1965), organic matter and minerals (Rao and Vebkaateswarly, 1982). The higher counts of *Azotobacter* under *A. nilotica* tree and nearly 25% increase in percent C in the soil suggested that organic matter may be an important factor in the occurrence of *Azotobacter*. Vancura et al (1965) have ascribed the low *Azotobacter* population of Egyptian soil to the high temperature which cause partial sterilization. Substrate availability i.e. amount of organic matter was also found to be a major limiting factor for the growth of free living nitrogen fixing bacteria (Stewart, 1969). Similarly, lower level of % C in *L. leucocephala* and lower count for free nitrogen fixer are reported in the present study.

REFERENCES

DOBEREINER, J. (1968). Non - simbiotic nitrogen fixation in tropical soils. Pesq. Agropec. Bras. 3: 1-6.

FELKER, P. (1979). Mesquite - an all purpose leguminous arid land tree. In: RITCHIE, G.A. (ed). New Agricultural Crops. Golden : Westview Press, p.89-132. (Am Assoc Adv of Sci Symp. Vol. 38).

JENSEN, H.L. (1965). Soil nitrogen. BARTHOLOMEW W.V and CLARK F.E. (eds). Amer. Asoc. Agron. Monogr. 10: 436-438.

MISHUSTIN, E.N. and SHILNIKOVA, V.K. (1969). The biological nitrogen by free - living bacteria. In \: Soil Biology. Paris : Unesco. p.65-124.

OLDEMAN, R.A.A. (1983). The design of ecologically sound agroforests. In: HUXLEY, P.A. (ed). Plant Research and Agroforestry. Nairobi : ICRAF. p. 173-206.

PCARR (1981). Leucaena. Promising forage and tree crop for the tropics. PHILIPPINE COUNCIL FOR AGRICULTURE AND RESOURCES RESEARCH AND U.S. NATIONAL ACADEMY OF SCIENCE.

RAO, A.V. and VEBKATESWARLU. B. (1982). Occurrence of Azotobacter and nitrogen fixation in the desertic soil. Indian J. Microbiol. 22: 255-257.

STEWART, W.D.P. (1969). Effect of substrate concentration on growth of Azotobacter. Proc. R. Soc. 172: 367-368.

TURVEY, N.D. and SMETHURST, P.J. (1983). Nitrogen fixing plants in forest plantation management. In: GORDON, J.C. and WHEERLER, C.T. (ed). Biological nitrogen fixation in forest ecosystems: Foundations and Applications. The Netherlands : Junk. p. 233-260.

VANCURA, V. Adbel, MALEK, Y. and ZAYED, M.N. (1965). Azotobacter population in Egyptian soil. Folia Microbiol. 10: 224-229.