Response of *Sesbania* **Green Manuring and Mungbean Residue Incor**poration on Microbial Activities for Sustainability of a Rice-Wheat Cropping System

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Abstract

The microbial population and its biomass and nitrogenase activity in plant roots under sandy-clay-loam soil conditions where the plant residues of *Sesbania rostrata* and mungbean (*Vigna radiata*) were incorporated were significantly higher than that obtained from summer fallow. The increases in yield of rice and wheat and the total nitrogen content in shoot and grain was also perceptible by the application of green manuring. The results highlight the importance of green manuring in rice-wheat cropping systems under tropical sandy-clay-loam soil conditions of India.

Keywords: green manuring, rice-wheat cropping system, microbial activities

1 Introduction

Rice-wheat system is the most wide-spread cropping system in each of the five Asian -Pacific regions. It covers about 22 million hectares in South-East Asia stretching over large areas in India, Pakisthan, Nepal, Bangladesh and China. In India it occupies about 10 million hectares and almost 73 per cent of the food requirement of the country is met by rice and wheat. This proportion is likely to increase to almost 77 per cent by the year 2010. Data from long term experiments, however, present a disturbing trend on sustainability of rice-wheat cropping system. The yield levels of both rice and wheat are either plateuing or registering a declining trend particularly in high pructivity regions of the country. There is a general decline in factor productivity and farmers have to use more and more fertilizer year after year to get the same level of yield as obtained with less amount of fertilizers in the previous years. This has been occurred due to decline in soil organic matter which led to impoverishment of soil fertility. Inclusion of a legume in cropping systems is an old established measure for recouping impoverished soil fetility. The usage and limitation of green manuring in low land rice has been reported (BECKER et al., 1995; BECKER and LADHA, 1997; BECKER, 2001). The soil microbial population is closely associated with organic matter of soil. Immediately after incorporation into soil, plant materials are subjected to the transformation and decomposition process of heterotrophic microflora (NEGI et al., 1986, 1987; RAUHE, 1987; SINGH and SINGH, 1993; TILAK et al., 1995). However, data on the effect of legumes, particularly when their residue is incorporated on biological properties of soil are, however, meagre. The present report deals with the effect of green manuring with Sesbania aculeata and

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S. rostrata and mungbean (*Vigna radiata*) residue incorporation on soil microbial population, microbial biomass, nitrogenase activity in roots, nitrogen content in shoot and grain and yields of straw and grain of rice and wheat crops.

2 Materials and Methods

2.1 Soil

A green house experiment was conducted with sandy-clay-loam soil having 52.5% sand, 21.0% silt and 26.5% clay, pH 8.2 (1:2:5 soil to 0.01M $CaCl_2$ solution), 0.60% organic carbon, 36 mg alkaline permanganate hydrolysable N per kg soil, 10 mg 0.5N $NaHCO_3$ extractable P per kg soil and 140 mg 1N ammonium acetate extractable K per kg soil.

2.2 Details of experiment

The experiment was laid out in cement pots having 20 kg of soil with five replications. The treatments were fallow, *Sesbania aculeata* green manuring, *S. rostrata* green manuring, mungbean (SR - both grain and residue harvested) and mungbean (SI -grain harvested and residue incorporated in soil) during summer (mid April - end June 2002).

Summer crops were seeded in the third week of April. Sesbania as well as mungbean received a basal dose of 20 kg N ha⁻¹ as urea and 17 kg P ha⁻¹ as single super phosphate. Fallow pots received no fertilizer during summer. Both species of *Sesbania* were grown to flowering (60 days) and then incorporated into soil as green manure. Mungbean variety P-16 was grown to maturity (65-70 days after sowing) and in one set of pots all aboveground vegetation was removed, whereas in second set of pots the matured pots were picked by hand and the remaining residue was incorporated into soil while preparing for rice transplantation.

For rice, the pots were flooded with water, tilled and puddled. 60 kg N ha⁻¹ as urea, 20 kg P ha⁻¹ as single super phosphate, 30 kg K ha⁻¹ as muriate of potash and 4 kg Zn ha⁻¹ as zinc sulphate hepta hydrate were applied to each pot. Three seedlings per hill of 25-30 days old of rice variety Pusa 169 were transplanted in the second week of July into each pot. Nitrogen was applied in two equal splits, 10 days and 30 days after transplanting rice. The plants were harvested in the last week of October.

For wheat, the pot was irrigated after harvesting rice and prepared for sowing. A basal dose of 40 kg N ha⁻¹ as urea, 20 kg P ha⁻¹ as single super phosphate and 30 kg ha⁻¹ as muriate of potash was applied. Three seedlings were allowed to grow in each pot. Wheat variety HD 2329 was sown in the last week of November and harvested in the third week of April.

2.3 Microbial assay

Microbial population and activities in soil were evaluated for each crop at 60 days of plant age. Three soil samples from 0-6 cm depth were collected from each replicate and composited. The soil was sieved through a 2 mm mesh screen after visible plant debris and fauna had been removed. Samples from different replicates were air dried

and analysed for various microbial counts by plating appropriate dilutions of soil solution in respective media and incubated at 30° C.

The bacterial and actinomycetes colonies were counted on soil extract agar (ALLEN, 1957) and fungal colonies on Rose Bengal agar medium (PARKINSON, 1973). The enumeration of *Azospirillum* was carried out by the most-probable-number (MPN) method using sodium malate semi-solid medium (DOBEREINER *et al.*, 1976). The MPN counts were calculated as per the tables outlined by ALEXANDER (1965). The phosphate solubilising bacteria (PSB) present in soil were counted by using a medium suspended with insoluble phosphate like tri-calcium phosphate. The production of clearing zones around the colonies is an indication of the presence of PSB (SUNDARARAO and SINHA, 1963). All microbial counts were expressed as $X \times 10^x$ per g of soil on air dry basis in each case.

The nitrogenase activity in root was estimated at 60 days of plant age by the acetylene reduction assay as described by TRIPATHI and KLINGMULLER (1992).

The soil microbial biomass C was estimated by fumigation extraction method (VANCE *et al.*, 1987). It was expressed as μ g per g oven dry soil (JENKINSON, 1988).

2.4 Plant parameters

The shoot biomass on dry weight basis and grain yield were recorded at the time of plant harvest. The total nitrogen content in shoot and grain was also determined after harvest by Kjeldahl method (PAGE *et al.*, 1982).

2.5 Statistical analyses

The data were analysed statistically as described by ${\rm Fischer}$ (1958). The critical difference at 5% significance level was calculated.

3 Results and Discussion

The role of soil microorganisms in sustainable productivity has been reviewed (LEE and PANKHURST, 1992; LATA *et al.*, 2000). Microbes including bacteria, fungi and actinomycetes are the principal decomposers of organic matter in soil. In the present study the population of bacteria, fungi, *Azotobacter* and *Azospirillum* was much larger during rice growth period than that during wheat growth period, whereas the population of actinomycetes was much higher during wheat growth period than during rice growth period. The population of phosphate solubilising bacteria (PSB) remained more or less similar during both the cropping periods.

Sesbania green manuring significantly increased the microbial population many fold over fallow; *S. rostrata* had more beneficial effect on microbial population than *S.aculeata*. As compared to fallow *S. aculeata* increased the population of bacteria, actinimycetes, fungi, *Azospirillum, Azotobacter* and PSB by 2.3, 3.7, 5.0, 3.2, 5.3 and 2.8 times, respectively during rice growth period and 1.7, 12.0, 1.7, 2.3, 2.0 and 2.5 times, respectively during wheat growth period, whereas *S. rostrata* green manuring increased population of bacteria, actinomycetes, fungi, *Azotobacter*, *Azospirillum* and PSB over fallow by

3.7, 16.3, 9.0, 4.8, 33.0 and 13.5 times, respectively during rice growth period and 2.3, 12.8, 3.0, 5.5, 75.0 and 3.0 times, respectively during wheat growth period. Growing mungbean also resulted in higher microbial population as compared to fallow and the results were quite encouraging when its residue was incorporated. It is interesting to note that the *Azospirillum* counts in soil was more in these soils than in others (Table 1). Reports indicate that green manuring with crimson clover (*Trifolium incarnatum*) in soil brought in significantly more number of *Bacillus* sp., actinomycetes and total culturable bacteria than did the soil from N fertilized, conventionally tilled treatment without green manuring (KIRCHNER *et al.*, 1993).

	Microbial population (per g soil)							
Treatment	Bacteria	Actinomycetes	Fungi	Azotobacter	Azospirillum	PSB		
	10 ⁵	10^{4}	10^{4}	10 ²	10 ⁴	10 ²		
Rice								
Fallow	42	0.3	0.1	22	0.9	0.4		
Sesbania aculeata	95	1.1	0.5	67	4.8	1.1		
Sesbania rostrata	155	4.9	0.9	105	29.7	5.4		
Mungbean (SR)	105	1.2	0.8	87	15.5	3.5		
Mungbean (SI)	167	5.5	1.3	202	37.5	6.0		
C.D. at 5%	40.5	1.25	0.72	25.8	10.7	0.9		
Wheat								
Fallow	32	0.4	0.3	11	0.01	2.1		
S. aculeata	55	4.8	0.5	25	0.02	5.2		
S. rostrata	75	5.1	0.9	60	0.75	6.3		
Mungbean (SR)	35	3.8	0.5	42	0.32	4.8		
Mungbean (SI)	67	9.5	0.8	68	0.62	5.1		
C.D. AT 5%	18.5	25.5	0.2	10.5	0.022	0.75		
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Table 1: Effect of summer crops and their residue on soil microbial population

SR: both grain and straw harvested; SI: grain harvested but straw incorporated

The microbial biomass in soil, in general, was more in 0-15 cm depth than in 15-30 cm layer. *S. rostrata* green manuring resulted in highest microbial biomass C followed by mungbean residue incorporation and *S. aculeata* green manuring (Table 2). Mungbean without residue incorporation has no added advantage over fallow which resulted in lowest microbial biomass at both depths of soil.

-	R	lice	Wheat		
Treatment	0-15 cm	15-30 cm	0-15 cm	15-30 cm	
Fallow	192.1	156.5	119.5	102.8	
S. aculeata	250.0	192.7	235.6	187.5	
S .rostrata	285.0	210.5	305.7	220.5	
Mungbean (SR)	200.5	155.5	205.8	105.8	
Mungbean (SI)	244.0	195.7	302.7	208.7	
C.D. at 5%	35.58	21.24	42.75	35.56	

 Table 2: Effect of summer crops on microbial biomass* at 0-15 cm and 15-30 cm soil depth.

The nitrogenase activity as determined by ARA technique revealed significant variations among the treatments. The activity was more in green manuring with *S. rostrata* and mungbean residue incorporation treatments (Table 3). Both the legumes are nodulating plant species and *S. rostrata* forms stem nodules in addition to root nodules. Contributions of legume-*Rhizobium* symbiotic system in augmenting soil fertility in various cropping systems have been well documented (CHALK, 1998). A number of nitrogenfixing bacteria including rhizobia are considered to be growth promoting rhizobacteria (PGPR). The effects of PGPR on plant growth can be mediated through the production of phytohormones or by supplying biologically fixed nitrogen (GLICK, 1995).

The yields of straw and grain were significantly more under green manuring treatments than under fallow. Green manuring wth *S. aculeata* and mungbean incorporation without residue resulted not much significant variation in straw and grain yield of rice and wheat. The differences between various amendments on dry shoot biomass and grain yield of wheat were not significant although they were superior to fallow treatment (Table 3). The total nitrogen content in shoot and grain at the time of harvest showed significant variations among various treatments (Table 4).

Management practices that conserve the biodiversity of microbial communities in marginal soils can sustain agricultural production over long periods of time. In this context, growing the green manure crops like *S. rostrata* or mungbean during rice-wheat cropping system is one of the ideal management practices for improving soil fertility and crop productivity. Rice and wheat being important cereal crops showing low productivity in marginal lands, these results will impart information for ameliorating soil quality and increase the unit land productivity. Legumes being an integral part of agriculture, the rhizobial partner and associated microflora in soil play a vital role in influencing soil quality and crop productivity.

Nitrogenase activity [‡] 5.2 28.5	Shoot dry biomass (g) 15.8 22.7	Grain yield (g) 4.8 9.5	Nitrogenase activity [‡] 6.2 30.7
-		-	-
28.5	22.7	9.5	30.7
102.8	30.5	11.5	85.6
32.5	25.7	8.7	35.8
86.5	27.5	10.2	75.7
2.55	5.75	4.58	3.47
	32.5 86.5	32.5 25.7 86.5 27.5	32.5 25.7 8.7 86.5 27.5 10.2

 Table 3: Effect of summer crops on shoot dry biomass, grain yield and nitrogenase activity of root of rice and wheat.

* per hill consisting of 5-6 fertile tillers

 † per three plants

 ‡ nitrogenase activity in root (n mol $\rm C_2H_4~h^{-1}g^{-1}$ root) of rice and wheat

Table 4: Effect of summer	crops on	total nitrogen	content in	shoot and	grain of rice
and wheat.					

	Total N content (mg per g dry weight)				
_	Ri	се	Wheat		
Treatment	Shoot	Grain	Shoot	Grain	
Fallow	3.5	11.8	4.2	13.5	
S. aculeata	4.7	12.5	5.9	15.4	
S. rostrata	7.5	14.5	9.2	19.8	
Mungbean (SR)	5.8	13.5	7.8	16.2	
Mungbean (SI)	6.5	14.7	9.2	18.5	
C.D. at 5%	0.95	0.58	1.55	1.25	

4 Summary

The microbial counts viz. bacteria, actinomycetes, fungi, *Azotobacter, Azospirillum*, PSB, the microbial biomass in soil and nitrogenase activity in roots were markedly higher in soil where green manuring with *Sesbania rostrata*, *S. aculeata* and green gram (*Vigna radiata*) was incorporated than in fallow soil. *Sesbania rostrata* brought 148.2 and 258.8 percent increase in microbial biomass over fallow treatment with rice and wheat,

respectively at 0-15 cm soil depth closely followed by mungbean residue incorporation in soil. Similar trend was noticed with the nitrogenase activity which was 19.8 and 13.8 times higher than the fallow soil with rice and wheat, respectively due to *S. rostrata* green manuring. This was reflected in better performance of rice and wheat as evidenced by the increase in dry shoot biomass, grain yield and total N content in shoot and grain at harvest under green house conditions. The increase in shoot dry biomass and grain yield due to *S. rostrata* green manuring over no amendment was 3.1, and 2.5 times, respectively with rice and 1.9 and, 2.4 times, respectively with wheat.

References

- ALEXANDER, M.; Most probable number method for microbial populations; in: Methods of Soil Analysis. Advances in Agronomy, Part II, edited by BLACK, E. A. et al.; pages 1467–1472; 1965.
- ALLEN, O. N.; Experiments in Soil Bacteriology; Burgess Publication Company, Minnesota, USA; 1957.
- BECKER, M.; Potential and limitation of green manure technology in low land rice; Journal of Agriculture in the Tropics and Subtropics; 102:91–108; 2001.
- BECKER, M. and LADHA, J. K.; Adaptation of green manure legumes to adverse conditions in rice low lands; *Biology and Fertility of Soils*; 23:243–248; 1997.
- BECKER, M., LADHA, J. K. and ALI, M.; Green manure technology : Potential usage and limitations. A case study for low land rice; *Plant and Soil*; 174:181–194; 1995.
- CHALK, P. M.; Dynamics of biologically fixed N in legume-cereal rotations: A Review; Australian Journal of Agricultural Research; 49:303–316; 1998.
- DOBEREINER, J., MARRIEL, I. E. and NERY, M.; Ecological distribution of *Spirillum lipoferum* Beijerinck; *Canadian Journal of Microbiology*; 22:1464–1473; 1976.
- FISCHER, R. A.; Statistical Methods for Research Workers, 13th Edition; Oliver and Boyd, London; 1958.
- GLICK, B. R.; The enhancement of plant growth by free living bacteria; *Canadian Journal of Microbiology*; 41:109–117; 1995.
- JENKINSON, D. S.; The determination of microbial biomass carbon and nitrogen in soil; in: Advances in Nitrogen Cycling Agricultural Ecosystems, edited by WILSON, J. R.; pages 368–386; CAB International, Wallingford, U.K.; 1988.
- KIRCHNER, M. J., WOLLUM II, A. G. and KING, L. D.; Soil microbial populations and activities in reduced chemical input agroecosystems; *Journal of Soil Science Society* of America; 57:1289–1295; 1993.
- LATA, SAXENA, A. K. and TILAK, K. V. B. R.; Biofertilizers to augment soil fertility and crop production; in: *Soil Fertility and Crop Production*, edited by KRISHNA, K. R.; pages 279–312; Science Publishers Incorporation, Enfield (NH) USA/ Plymouth UK; 2000.
- LEE, K. E. and PANKHURST, C. E.; Soil organisms and sustainable productivity; Australian Journal of Soil Research; 30:855–892; 1992.
- NEGI, M., SADASIVAM, K. V. and TILAK, K. V. B. R.; Dynamics of microbial population in the rhizosphere of barley (*Hordeum vulgare*) as influenced by inoculation with *Azotobacter chroococcum* and *Azospirillum brasilense* in organic amended soils;

Indian Journal of Microbiology; 26:56-61; 1986.

- NEGI, M., SADASIVAM, K. V. and TILAK, K. V. B. R.; A note on the effect of nonsymbiotic nitrogen fixers and organic wastes on yield and nitrogen uptake of barley; *Biological Wastes*; 22:179–185; 1987.
- PAGE, A. L., MILLER, R. H. and KEENCY, D. R.; Methods of Soil Analysis, Part II Chemical and Microbiological Properties, Second Edition; Agronomy No. 9; American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin USA; 1982.
- PARKINSON, D.; Techniques in the study of soil fungi; *Bulletin of Ecology Research Committee, Stockholm*; 17:29–36; 1973.
- RAUHE, K.; Effects of organic manuring and cropping on soil humus and fertility; in: *Agricultural Waste Management and Environmental Protection*, edited by WALTE, E. and SZABOLES, I.; 4th International CIEC Symposium Proceedings ,International Science Centre of Fertilizers, Belgrade; 1987.
- SINGH, H. and SINGH, K. P.; Effect of residue placement and chemical fertilizer in soil microbial biomass under tropical dry land cultivation; *Biology and Fertility of Soils*; 16:275–281; 1993.
- SUNDARARAO, W. V. B. and SINHA, M. K.; Phosphate dissolving organisms in the soil and rhizosphere; *Indian Journal of Agricultural Sciences*; 33:272–278; 1963.
- TILAK, K. V. B. R., SAXENA, A. K. and DATT, N.; Dynamics of microbes in agricultural soil with different management practices; *Journal of Soil Biology and Ecology*; 15:117–126; 1995.
- TRIPATHI, A. K. and KLINGMULLER, W.; Temperature sensitivity of nitrogen fixation in *Azospirillum* sp.; *Canadian Journal of Microbiology*; 3:1238–1241; 1992.
- VANCE, E. D., BURKES, P. C. and JENKINSON, D. S.; An extension method for measuring soil microbial biomass C; Soil Biology and Biochemistry; 20:329–335; 1987.