Indian Journal of Weed Science **50**(3): 282–286, 2018

Print ISSN 0253-8040 Online ISSN 0974-8164

Dynamics of microbial community and enzyme assay as influenced by green manuring and weed control measures in rice-groundnut cropping system

S.S. Pinjari*, S.B. Ganagawane, Y.R. Govekar, U.V. Mahadkar and N.A. Meshram

Department of Agronomy, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra 415 712

**Email: pinjari94222@gmail.com*

Article information

DOI: 10.5958/0974-8164.2018.00060.6

Type of article: Research article

Received : 2 June 2018
Revised : 29 July 2018
Accepted : 2 August 2018

Key words
Bacteria

Fungi

Free living nitrogen fixers

Microbial biomass carbon

Phosphate solubilizers

ABSTRACT

A long-term experiment was commenced (2011) and conducted at the Research Farm of Dapoli (Maharashtra) during *Kharif* and *Rabi* season in rice–groundnut cropping system, to evaluate the effect of green manuring and different weed control measures on the survival and growth of total bacteria, total fungi, total free living nitrogen fixers and total phosphate solubilizers in rhizospheric soil. Results emerged out from the conduct during 2011 to 2014 indicated that, green manuring (in-situ application of *Sesbania rostrata* 45 DAS) and without green manuring (control) as a main plot treatment and among the weed control measures and sub plot treatments comprising, comparative effects of hand weeding, fixed herbicide pretilachlor (pre-emergence) for rice crop and pendimethalin (pre-emergence) for groundnut crop, and different rotational herbicides (for rice crop, pyrazosulfuron 0.030 kg/ha at 8-10 DAT (1 year)**,** fenoxaprop -p-ethyl 0.056 kg/ha at 25-30 DAT (2 year)**,** oxadiargyl 0.100 kg/ha at 0-5 DAT (3 year), and for groundnut crop oxadiargyl 0.12 kg/ha at 0-2 DAS-1 year, butachlor 1.0 kg/ha at 0-3 DAS-2 year, alachlor1.5 kg/ha at 0-3 DAS-3 year) application to both the crops were significantly tested along with weedy check. The results concluded that the green manuring significantly increased in microbial populations than without green manuring. There were no adverse effects of herbicidal use on all the estimated microbial population at all the stages of both the crops. In contrast to use of fixed herbicide pretilachlor-S 0.75 kg/ha for rice and pendimethalin 1.00 kg/ha and different rotational herbicides had no long-term adverse effects on rhizosphere micro-flora of rice–groundnut cropping system.

INTRODUCTION

Soil micro-organisms and soil enzymes play a major role in soil fertility as these involve in the cycling of nutrients like carbon, nitrogen, phosphorus and sulphur, which are required for the plant growth. They are the sensitive biological indicators of soil quality evaluation because they can sensitively reflect minute changes in the soil environment. Soil microbial biomass is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Bamboo *et al*. 2013). Healthy population of bacteria, fungi and actinomycetes can stabilize the ecosystem. Any change in the population and activity may indirectly affect the nutrient cycling, which in turn affects the productivity, fertility and other soil functions (Wang *et al*. 2008).Soil enzymes, the vital activators in life processes, are known to play a substantial role in maintaining the soil health and its environment. They are important in catalyzing several vital reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick, 1997).

Soil enzymes provide a unique integrative biological assessment of soil function, especially those that catalyze a wide range of soil biological processes, such as dehydrogenase, urease and phosphatase (Nannipieri *et al*. 2002). The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). Phosphatase catalyzes hydrolytic break down of phosphor monoesters, thereby showing a high correlation between the content of soil phosphorus and an indicator of soil fertility. Urease is the enzyme

that catalyzes the hydrolysis of urea to CO_2 and NH_4^+ ions byacting on C-N non-peptide bonds in linear amides. It is an important enzyme in soil that mediates the conversion of organic nitrogen to inorganic nitrogen and has been widely used to evaluate the changes in soil fertility (Nazreen *et al*. 2012). Herbicide usage seems to be inevitable in transplanted rice, since weeds are the prime biological constraint due to the simultaneous emergence of rice and weed seedling, scarcity of labour and huge labour cost. A large numberof pre- and post-emergence herbicides are used by the farmers to control weeds in rice.

The pre- and post-emergence application of herbicides resulted in large proportion of herbicides reaching the soil and accumulating in top 0 to15 cm depth causing ecological damage (Latha and Gopal 2010). Preferred herbicides should not only have good efficacy, but also pose minimum adverse effects to crop, ecology and environment (Constenla *et al*. 1990). The continuous use of herbicides with similar mode of action might lead to the development of resistance in certain weeds to the herbicides and cause shift in weed flora. One of the recent advent ways to overcome this problem is the use of different herbicide. The impact of different herbicides on soil enzymatic activity and microbial population has not been studied so far. Hence, a study was conducted with an objective to find out the impact of herbicide on bacteria, fungi, nitrogen fixer, phosphate solubilizers, microbial biomass carbon and basal soil respiration in rice–groundnut cropping system

MATERIALS AND METHODS

The field experiment was started on 2011 and conducted for four years study at the Research Farm of All India Co-ordinated Research Project on Weed Management under Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (Maharashtra) for rice-groundnut cropping system. The experiment was conducted on *Kharif* rice (Ratnagiri-24) and *Rabi* groundnut (KonkanTapora) cropping system, which included eight treatment combinations laid out in a split plot design (SPD) with three replications. The main plot treatments included green manuring, *viz*. green manuring with *Sesbania rostrata* (in-situ application after 45 DAS) and without green manuring (control) while the sub-plot treatments included weed control measures such as hand weeding at 20 and 40 DAS, fixed herbicide pretilachlor-S 0.75 kg/ha 3-7 days after transplanting (DAT) for rice crop and pendimethalin 1.0 kg/ha PE (pre-emergence) for groundnut crop and different rotational herbicides (for rice crop, pyrazosulfuron 0.030 kg/ha at 8-10 DAT(1 year)**,** fenoxaprop-p-ethyl 0.056 kg/ha at 25-30 DAT (2 year)**,** oxadiargyl 0.100 kg/ha at 0-5 DAT (3 year), and for groundnut crop oxadiargyl 0.12 kg/ha at 0-2 DAS-I year,butachlor 1.0 kg/ha at 0-3 DAS-2 year, alachlor1.5 kg/ha at 0-3 DAS-3 year), weedy check. Rhizospheric soils were collected at 30, 50 days after sowing (DAS) and at harvesting stage of both the crops by uprooting of four plants from each plot and keeping the soil around root system intact. After removing the bits of plant roots and other debris, the soil strongly adhered to the roots was immediately used without drying for determination of biological property of rhizospheric soils.

The total bacterial population, total fungal population, free living nitrogen fixers and total phosphate solubilizers of rhizosphere soil were determined. The colony forming units (CFU) of bacteria were enumerated on nutrient agar-agar media by serial dilution technique for bacterial populations, Martin's rose Bengal agar media used for total fungal population (Martins 1950), Noories N-free media used for total free living nitrogen fixers (Noories 1959) and Pikovskaya's agar medium used for total phosphate solubilizing microorganisms (Pikovskaya 1948). The relative equivalent yield (REY) was calculated by the mixture yields of a component crop expressed as a portion of its yields as a sole crop from the same replacement series is the relative yield of crop and sum of the relative yields of component crop. The experimental data were subjected to analysis of variances (ANOVA) and treatment means were compared, significant differences were tested at p=0.05 using split plot design (SPD) as given by Panse and Sukhatme (1985) using computer design DBSKKV_STAT.

RESULTS AND DISCUSSION

Effect of green manuring

The populations of all soil microbes were significantly influenced due to green manuring as compare to without green manuring (**Tables 1-4**). Significantly higher microbial population were found in green manuring treatment as compared to the without green manuring at 30 DAS, 50 DAS and at harvesting stage in *Kharif* rice as well as in *Rabi* groundnut. The increase in microbial population with the incorporation of green manuring (sesbania) in soil due to addition higher biomass reflects higher microbial diversity in soil (Tilak 2004). The green manures applied to the soils, it increases soil organic matter which helps to increased microbial populations, mineralization, enzymes assay and also maintain the soil C/N ratio (Tejada *et al*. 2008).

Effect of weed control measure

In rice crop, the treatment weed free check (2 HW) significantly influenced all soil microbes' population except fungi, which were found to be significantly superior over fixed herbicide, rotational herbicide and weedy check at 30 DAS, 50 DAS and at harvesting stage. The bacterial populations were also found significantly less in fixed herbicide and the rotational herbicide as compared to weedy check at 30 DAS, which were found to be at par with weedy check at 50 DAS and at harvest (**Table 1 and 2**). In respect of groundnut crop, the treatment of hand weeding twice caused significant increase in population of all soil microbes (*viz*. bacteria, nitrogen fixers, phosphate solubilizers, microbial biomass, basal soil respiration) except fungi population, which were noticed significantly superior than the both fixed and rotational herbicide and remain at par with weedy check at initial stage of 30 DAS, (**Table 3 and 4**). Microorganisms are able to degrade herbicides and utilize them as a source of biogenic elements for their own physiological processes. However, before degradation, herbicides have toxic effects on microorganisms, reducing their abundance, activity and consequently, the diversity of their communities (Adhikary *et al*. 2014). The toxic effects of herbicides are normally most severe immediately after application, when their concentrations in soil are the highest. Later on, microorganisms take part in a degradation process, and herbicide concentration and its toxic effect gradually decline up to half-life. Then the degraded organic herbicide provides the substrate with carbon, which leads to an increase of the soil microflora (Bera and Ghosh, 2013, Goveykar *et al*. 2014, Kumar 2015).The interaction effects of green manuring and weed control measures were found to be statistically non-significant.

Total relative equivalent yield of rice–groundnut cropping system

Effect of green manuring

The REY of rice-groundnut cropping system did not influence due to green manuring in whole pooled results. However, green manuring was recorded higher total REY of rice-groundnut cropping system than without green manuring (**Table 5**).

Table 1. Combined effects of green manuring and weed control measure on bacterial, fungal population and nitrogen fixers in rice–groundnut cropping system during *Kharif* season

Treatment	Bacteria (CFU x10^{-6}/g of soil)			Fungi (CFU x10^{-4}/g of soil)			Nitrogen fixers (CFU x10^{-3}/g of soil)		
	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest
Main plot treatment : Green manuring									
Green manuring	39.63	36.04	32.69	26.43	22.96	19.76	28.86	24.71	22.25
Without green manuring	31.85	31.49	26.67	17.32	17.10	12.97	21.23	20.34	17.16
LSD (p=0.05)	2.71	0.76	3.50	5.76	2.07	2.82	2.92	2.24	1.85
Sub plot: Weed control measures									
Fix herbicide – pendimethalin(PE)	33.62	31.82	27.03	20.39	19.03	15.99	22.85	20.54	17.33
Rotational herbicide – alachlor	33.80	31.63	27.17	20.07	18.35	15.17	22.62	20.45	17.65
Weed free check	38.98	37.72	34.38	24.92	22.64	18.27	28.94	26.64	24.15
Weedy check	36.56	33.88	30.12	22.10	20.09	16.03	25.75	22.47	19.70
LSD (p=0.05)	2.13	2.13	3.06	NS	NS	NS	2.02	2.95	4.10

Table 2. Combined effects of green manuring and weed control measure on phosphate solubilizers, microbial biomass carbon and basal soil respiration in rice – groundnut cropping system during *Kharif* season

Treatment	Phosphate solubilisers (CFU x10^{3}/g of soil)			Microbial biomass carbon (µg/g of soil)			Basal soil respiration (µg CO_2/100 g of soil)		
	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest
Main plot treatment : Green manuaring									
Green manuring	27.87	23.57	20.78	234.74	220.29	210.48	238.74	227.54	208.63
Without green manuring	19.38	18.74	15.55	199.32	204.70	192.71	209.22	205.51	194.78
LSD (p=0.05)	2.25	1.34	2.43	10.17	6.36	0.68	12.60	1.20	4.19
Sub plot: Weed control measures									
Fix herbicide - pendimethalin(PE)	21.37	19.11	16.28	210.42	206.71	197.21	215.03	209.50	196.22
Rotational herbicide – alachlor	21.29	19.29	16.45	208.67	204.63	195.03	216.06	209.37	196.46
Weed free check	28.14	26.11	22.54	230.49	225.96	213.08	238.36	231.36	212.05
Weedy check	23.71	20.12	17.41	218.54	212.68	201.08	226.46	215.89	202.09
LSD (p=0.05)	2.67	3.00	3.01	4.90	9.73	9.96	10.05	7.12	6.00

Note: Interaction between green manuring and weed control measures were non-significant during all the stages of observations

Table 3. Combined effects of green manuring and weed control measures on soil bacterial, fungal population and nitrogen fixers in rice-groundnut cropping system during *Rabi* season

Treatment	Bacteria (CFU × 10^{-6}/g of soil)			Fungi (CFU ×10^{-4}/g of soil)			Nitrogen fixers (CFU ×10^{-3}/g of soil)		
	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest
Main plot treatment : Green manuring									
Green manuring	44.72	48.49	43.57	29.10	32.18	28.84	28.01	30.99	26.57
Without green manuring	38.87	42.16	36.54	25.09	28.41	21.64	23.56	26.59	22.53
LSD (P=0.05)	1.50	1.53	1.44	2.80	1.53	2.49	2.51	3.35	2.08
Sub plot: Weed control measures									
Fix herbicide – pendimethalin(PE)	39.57	42.94	37.57	25.19	28.77	23.97	23.89	27.53	22.54
Rotational herbicide – alachlor	39.56	43.40	37.99	26.35	29.97	23.91	25.11	28.07	23.41
Weed free check	45.88	49.09	43.91	29.03	32.15	27.02	28.06	30.98	27.00
Weedy check	42.19	45.87	40.75	27.81	30.29	26.07	26.08	28.59	25.25
LSD (P=0.05)	3.67	NS	NS	NS	NS	NS	2.13	NS	NS

Note: Interaction between green manuring and weed control measures were non-significant during all the stages of observations

Table 4. Combined effects of green manuring and weed control measures on phosphate solubilizers, microbial biomass carbon and basal soil respiration in rice-groundnut cropping system during *Rabi* season

Treatment	Phosphate solubilisers (CFU × 10^{-3}/g of soil)			Microbial biomass carbon (µg/g soil)			Basal soil respiration (µg CO_2/100 g soil)		
	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest	30 DAS	50 DAS	Harvest
Main plot: Green manuring									
Green manuring	27.91	29.94	26.58	245.37	265.05	244.67	236.55	259.43	238.84
Without green manuring	23.87	26.29	21.78	219.38	244.23	222.69	215.99	231.93	218.25
LSD (P=0.05)	1.23	2.69	2.24	14.56	12.12	17.54	5.49	18.37	13.95
Sub plot: Weed control measures									
Fix herbicide – pendimethalin(PE)	24.17	26.52	22.64	222.46	247.66	228.10	219.52	240.52	223.80
Rotational herbicide – alachlor	24.70	27.42	22.98	224.42	251.54	229.23	221.60	241.30	224.61
Weed free check	28.32	30.37	26.87	248.00	263.00	242.29	236.45	253.67	236.53
Weedy check	26.38	28.14	24.23	234.62	256.36	235.12	227.51	247.23	229.23
LSD (p=0.05)	2.66	NS	NS	8.65	NS	NS	7.74	NS	NS

Note: Interaction between green manuring and weed control measures were non-significant during all the stages of observations

Table 5. Effects of green manuring and weed control measures on relative equivalent yield of groundnut and total relative equivalent yield of rice-groundnut cropping system

Treatment	REY of Groundnut (t/ha)					Total REY of system (t/ha)				
	2011	2012	2013	2014	Pooled	2011	2012	2013	2014	Pooled
Main plot treatment : Green manuring										
Green manuring	16.72	15.08	14.10	13.65	13.47	20.87	20.68	17.67	17.81	17.84
Without green manuring	15.73	14.78	13.98	13.45	13.10	18.39	19.00	17.37	17.45	16.67
LSD (p=0.05)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sub plot: Weed control measures										
Fixed. herbicide – pendimethalin(PE)	17.02	15.68	14.61	14.16	13.90	20.51	20.55	17.88	18.19	17.82
Rotational herbicide – alachlor	14.45	15.28	13.92	13.65	12.95	18.44	20.12	17.26	17.43	16.95
Weed free check	18.00	17.07	16.03	15.48	15.05	21.70	22.34	19.67	20.39	19.44
Weedy check	15.44	11.70	11.61	10.91	11.23	17.87	16.35	15.25	14.51	14.81
LSD (p =0.05)	N.S.	2.19	0.60	2.07	1.13	2.64	2.13	0.86	2.12	1.27

Note: Interaction between green manuring and weed control measures were non-significant during all the stages of observations

Effect of weed control measures

As regard to the total REY of system, weed free check was recorded significantly higher yield over rest of the treatments during the years 2013, 2014 as well as in pooled results, while it was on the same level with fixed herbicide during the years 2011 and 2012. Among the use of fixed and rotational herbicide, significantly higher total REY of the rice-groundnut cropping system were obs recorded over weedy check, which remained at par with each other. The interaction effect between green manuring and weed control measures were found to be non-significant during all the years of experimentation and in pooled results. Effective weed control along with

higher total REY of the system by incorporation of green manuring to *Kharif* rice and application of pre emergence herbicide for rice and groundnut are confirmative with the present investigation. Singh *et al*. (2009) noticed that under dry seeding, higher grain yield was recorded with pre- emergence application of pendimethalin 1.50 kg/ha. The difference in yield might be due to differences in application mode and efficacy of herbicides against weed species. Similar results were obtained by Kaur and Singh (2015) under sequential use of pre- and post-emergence herbicides, resulting in more equivalent grain yield and net returns.

It can be concluded that the green manuring management practices stimulated significantly higher microbial soil health in respect to soil microbial populations, enzyme activities, soil microbial biomass carbon, CO_2 evolution due to maximum contribution of organic matter as compared to the without green manuring in both the crops. The microbial soil indicators were not adversely affected by herbicides during all the stages of the *Rabi* groundnut and initially suppressed due to toxic effect of herbicides at initial stage (30 DAT) in rice crop during *Kharif* season. In option to getting higher productivity, application of fixed herbicide improves the total REY of the system.

REFERENCES

Adhikary P, Shil S and Patra PS. 2014. Effect of herbicides on soil microorganisms in transplanted chilli. *Global Journal of Biology, Agriculture and Health Sciences* **3**(1): 236–238.

Bamboo N, Pasyat M, Samal A, Kujur M, Maharana JK and Patel AK. 2013. Effect of four herbicides on soil organic carbon, microbial biomass-C, enzyme activity and microbial population in agricultural soil. *International Journal of Environmental Science and Technology* **3**: 100–112.

Bera S and Ghosh RK. 2013. Soil physico-chemical properties and microflora as influenced by bispyribac-sodium 10% SC in transplanted *Kharif* Rice. *Rice Science* **20**(4): 298–302.

Burns RG. 1978. Enzyme activity in soil: Some theoretical and practical considerations. pp. 295–340. In: Soil Enzymes, Burns, R.G. (Ed.). Academic Press, London.

Constenla MA, Riley D, Kennedy SH, Rojas CE, Mora LC and Stevans, JEB. 1990. Paraquat behaviour in Costa Rican soils and residues in coffee. *Journal of Agriculture Food Chemistry* **38**: 1985–1988.

Dick RP. 1997. Soil enzyme activities as indicator of soil quality. pp. 121–56 In: *Biological Indicators of Soil Health*, (Eds. Pankhurst *et al.*). CAB International, Wellingford.

Govekar YR, Mahadkar UV, Dahiphale AV Pawar LG, Nevase VB, Mane MJ and Gosavi SP. 2014. Effects of different tillage systems and herbicide on soil microflora of Lablab bean rhizosphere. *Indian Journal of Weed Science* **46**: 370–372.

Kaur Simerjeet and Surjit Singh. 2015. Bio-efficacy of different herbicides for weed control in direct-seeded rice. *Indian Journal of Weed Science* **47**: 106–109.

Kumar Rajinder, Dinesh Badiyala, Neelam Sharma and Suresh Gautam. 2015. Effect of long-term application of herbicides on soil microbial demography in rice-wheat cropping sequence. *Indian Journal of Weed Science* **47**: 71–74.

Latha, PC and Gopal H. 2010. Effect of herbicides on soil microorganism. *Indian Journal of Weed Science* **42**: 217–22.

Martin JP. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Science* **69**: 215.

Nannipieri P, Kandeler E and Ruggiero P. 2002. Enzyme activities and microbiological and biochemical process in soil. pp. 1–33. In: Enzymes in the Environment, (Eds. Burns RG and Dick RP). Marcel Dekker, New York.

Nazreen C, Mohiddin J, Srinivasulu M, Rekhapadmini A, Ramanamma P and Rangaswamy V. 2012. Interaction effect on insecticides on enzymatic activities in black soil from groundnut (Arachishypogaea L.) field. *Environment Research and Engineering Management* **2**: 21–28.

Noories JR. 1959. Isolation and identification of isolate Azotobacter. *Laboratory Practices* **8**: 239–240.

Panse VG and Sukhatme PV. 1985. *Statistical Methods for Agricultural Workers*, ICAR, New Delhi.

Pikovskaya RI. 1948. Mobilization of phosphorous in soil in connection with vital activity by some microbial species. *Microbiologia* **17**: 362–370.

Singh B, Malik RK, Yadav A and Nandal DP. 2009. Herbicide efficacy in seeded rice with different methods under wet and dry conditions. *Indian Journal of Weed Science* **41**: 37–40.

Tejada M, Gonzalez JL, García-Martínez AM and Parrado J. 2008. Effects of different green manures on soil biological properties and maize yield. *Bioresource Technology* **99**: 1758–1767.

Tilak KVBR. 2004. Response of Sesbania Green Manuring and Mungbean Residue Incorporation on Microbial Activities for Sustainability of a Rice-Wheat Cropping System. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* **105**: 189–196.

Wang QK, Wang SL and Liu YX. 2008. Response to N and P fertilization in a young Eucalyptus dunniiplantation: Microbial properties, enzyme activities and dissolved organic carbon. *Applied Soil Ecology* **40**: 484–90.