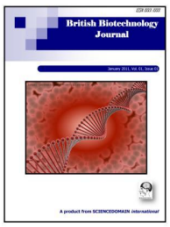

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Study of Microbial Population and Enzyme Activities in Intercropped Peanut Rhizosphere with Different Nutrient Application

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Research Article

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ABSTRACT

The aim of this study was to investigate the effect of different levels of chemical fertilizers (CF) alone or in combination with farmyard manure (FYM) under lime or no lime amelioration on biological properties of the rhizosphere soil of peanut, grown as intercrop with sabai grass (*Eulaliopsis binata* (Retz.) C.E. Hubb) in an acid lateritic soil. The effect of five fertilization levels viz., no CF (F_0), CF @ 20:40:30 (F_1), CF @ 40:80:60 (F_2) kg ha^{-1} NPK, F_1 + 2.5 t FYM ha^{-1} (F_3) and F_2 + 5 t FYM ha^{-1} (F_4) with (2 t ha^{-1}) and without lime application were studied on the bacterial density, dehydrogenase and phosphatase enzyme activities (i.e. acid and alkaline phosphomonoesterases), nitrogen accumulation in nodules and phosphorus solubilizing power at 25, 50, 75 and 100 days after sowing of peanut for two years. Results showed significant effects of fertilization levels and growth stages of the crop on the microbial activities. Populations of symbiotic nitrogen fixing and phosphorus solubilizing bacteria, soil enzyme activities, nitrogen accumulation in nodules and phosphorus solubilizing power in the FYM+CF treated plots significantly increased compared to sole CF treatments under both lime and no lime application. Lime application improved the activity of dehydrogenase and alkaline phosphomonoesterase enzymes, while decreased acid phosphomonoesterase activity. This study revealed that integrated application of optimum level of inorganic fertilizer, farmyard manure along with lime could improve the biological properties of an acid lateritic soil as well as the growth of peanut under sabai grass-peanut intercropping system.

Keywords: Arachis hypogaea; dehydrogenase; phosphomonoesterases; N fixation; P solubilization; acid soil;

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1. INTRODUCTION

The natural ability of soil to support optimum growth and yield of crops depends not only on its physical and chemical properties but also on the intensity of biological processes. Soil fertility is in great extent controlled by different biochemical activities of the microflora especially in the immediate surroundings of the roots, the rhizosphere, which under the influence of roots, carry a particularly dense population of microorganisms. Being a living part of soil organic matter, they also play an important role in the availability and recycling of soil nutrients and in the nutrient storage capability of soils (Arancon et al., 2006). Microorganisms can alter the degree of the nutrient supply to higher plants through decomposition of organic compounds, immobilization of available nutrients, mineralization of nutrients or by promoting the solubilization of fixed or insoluble mineral forms. The enzymatic activities of a soil catalyzes the biochemical activities performed by bacteria (Sarapatka, 2003) and thereby indicates the potential of the soil to permit the basic biochemical processes necessary for maintaining soil fertility. Dehydrogenase activity is an estimation of overall microbial activity due to its presence in all microorganisms (Taylor et al., 2002). Phosphatase activity is important as it is related to phosphorus cycle in the soil (Aon and Colaneri, 2001). Therefore, while studying the effect of different levels and sources of fertilization on soil fertility, attention must be focused to the activity of the microflora towards soil biological quality. In the present investigation an attempt has been made to examine the influence of different levels and sources of fertilization on the soil bacterial population, activity of enzymes, microbial activities and growth of peanut under sabai grass-peanut intercropping system in acid lateritic soil.

Sabai grass (*Eulaliopsis binata* (Retz.) C.E. Hubb), a perennial plant, contains about 54.5% of cellulosic material and high quality fiber (MacDonald and Franklin, 1969), which makes it an excellent raw material for paper pulp industries, only next to bamboo (Mohapatra et al., 2001). Sabai grass consists of long leaf fibres, high cellulose and pectose with low lignin content. For paper making, sabai is reputed to be superior to most other available grasses. The fibre length of sabai grass is more than that of bamboo but fibre diameter is 2.5-times lower than bamboo (Dutt et al., 2004). It is used where high strength is not required. It can also be used as a filler material in plastics and in mud matrix after suitable pretreatment to the surface. Understanding the structure, mechanical and thermal behavior of this grass fibre will open up new avenues for the utilization of this grass fibre (Chand and Rohatgi, 1992).

Because of flexibility, luster and strength properties of the leaves, it is used for making ropes and other rope based utility items (Mohapatra et al., 2001), for which sabai grass has an important role in tribal economics of many regions of India (Anonymous, 2002a) as well as many Asian countries like China, Pakistan, Nepal, Bhutan, Mayanmar, Thailand, Malaysia and Philippines (Yong, 1994). Wide spacing and initial slow growth rate of sabai grass provides ample scope for intercropping in association with legumes in the initial 1-2 years (Mohapatra et al., 2001; Basu et al., 2006a, 2006b, 2011, Mahapatra, 2011). Higher yield and net return from a sabai grass-peanut and sabai grass-blackgram intercropping systems over the sole sabai grass has been reported by Basu et al. (2006).

Among all the oilseed crops, peanut (*Arachis hypogaea* L.) has the first place in India (Basu, 2011) accounting more than 28% acreage and 32% production in the country (Anonymous, 2004). In the eastern India sabai grass is mainly grown in the acid lateritic uplands, which are recognized as low productive problem soils due to low organic matter and nitrogen (N) status, poor availability of phosphorus (P) and retention capacity for both moisture and nutrients (Beckie and Ukrainetz, 1996; Anonymous, 2002b). Therefore, appropriate level and

sources of nutrients can improve the soil fertility in terms of physical, chemical and biological properties and thereby promote optimum plant growth and maximize crop yield. Information related to the effect of fertilization on the soil biological properties in the rhizosphere of legume under sabai grass-legume intercropping system till now has not been reported in scientific literature.

Therefore, the present investigation aims to study the biological properties of rhizosphere soil of peanut, intercropped with sabai grass, as influenced by different fertilization treatments in the acid lateritic uplands of Eastern India.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The research farm is situated in the lateritic belt of south-western region of West Bengal, India, which is intersected by $22^0 19^/$ North latitude and $87^0 19^/$ East longitude at a distance of 115 km from the Bay of Bengal and has an elevation of 44.0 m above the mean sea level. The climate of the region is warm and humid. The average annual rainfall is 1400 mm, about 80% of which is received from mid June to mid October.

The soil is acid lateritic (Haplustalf) having sandy loam texture with 61% sand, 21% silt and 18% clay. Coarse and shallow depth of the soil results in low plant available water holding capacity causing moisture stress during crop growing period. The detailed physical, chemical and biological properties of the experimental soil are presented in Table 1.

Table 1. Physical, chemical and biological properties of experimental soil (0-20 cm)

Particulars	Value
Sand (%)	61.3
Silt (%)	21.4
Clay (%)	17.3
pH (1 : 2.5:: Soil : Water)	5.20
Organic carbon (g kg^{-1})	2.9
Total N, %	0.049
Available N, mg kg^{-1}	65.60
Total P, %	0.039
Available P, mg kg^{-1}	5.12
Total K, %	0.058
Available K, mg kg^{-1}	44.53
Population of P solubilizing bacteria (log no. CFU g^{-1} soil)	4.62
Dehydrogenase enzyme activity (µg TPF g^{-1} soil h^{-1})	6.99
Acid phosphatase enzyme activity (µg PNP g^{-1} soil h^{-1})	178.3
Alkaline phosphatase enzyme activity (µg PNP g^{-1} soil h^{-1})	41.2
P solubilizing power (mg insoluble P solubilized 15 mg^{-1} insoluble P g^{-1} soil)	0.045

2.2 TREATMENTS

The experiment was conducted for three years during 2003-'04, 2004-'05 and 2005-'06 at the fallow uplands of experimental farm in Indian Institute of Technology, Kharagpur, India. The treatments were consisting of five levels of fertilization viz., No fertilizer (F_0), chemical fertilizer (CF) @ 50:25:25 (F_1), CF @ 100:50:50 (F_2) kg ha^{-1} N, P and K respectively, farmyard manure (FYM)+CF @ 50 kg (F_3) and 100 kg (F_4) N ha^{-1} and these were tested under two levels of lime viz., no lime (L_0) and lime @ 2 t ha^{-1} (L_1). In F_3 and F_4 treatments FYM was applied to supply half of the N dose from F_1 and F_2 levels and the balance amount of N, P and K was supplied through CF. All together 10 treatment combinations with three replications were laid out in factorial completely randomized block design. The same experiment was continued for consecutive three years with fresh application of similar treatment combinations.

The effect of these treatments was studied on sabai grass-peanut intercropping system during wet season of 2003 and 2004 and on sole sabai grass during wet season of 2005. Peanut was grown with sabai grass as an intercrop at 1:2 ratio (two rows of peanut after every row of sabai grass) in the initial two years, since smothering effect due to vigorous growth of sabai grass did not allow growing peanut in the subsequent year. In this paper the effect of treatments on performance of peanut has been presented.

Lime and FYM were thoroughly incorporated in top 20 cm soil using power tiller and spade during 30 and 15 days before planting/sowing of crops respectively as per the treatments. Urea, single super phosphate and muriate of potash were used as the sources of inorganic fertilizers for supplying N, P and K respectively. Total quantity of single super phosphate and muriate of potash and half of urea was applied as basal dose at the time of sowing/planting/regrowth of crops during wet season. Remaining half of urea was top dressed in equal split through side dressing to sabai grass at 30 and 60 days after planting or regrowth.

Since sabai grass is perennial in nature 10-12 slips per hill were planted once during rainy season of 2003 (July) at a spacing of 100 cm x 50 cm for row-to-row and plant-to-plant respectively. Seeds of peanut were sown with a spacing of 30 cm x 10 cm for row-to-row and plant-to-plant respectively. Data on different growth parameters of peanut viz., number of branches per plant, leaf area index (LAI) and nodule number per plant were recorded at 50 days after sowing (DAS) and dry matter production was recorded at 25, 50, 75 and 100 DAS from randomly selected 10 plants.

2.3 SAMPLING OF SOIL

Rhizosphere soils were collected at different growth stages of peanut (25, 50, 75 and 100 DAS) by uprooting 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 soil biological properties. The population of symbiotic N fixing bacteria and P solubilizing bacteria, activity of dehydrogenase, acid phosphatase and alkaline phosphatase enzymes, nitrogen accumulation in nodules and phosphorus solubilizing power of rhizosphere soils were determined.

2.4 VIABLE COUNT OF BACTERIA

The colony forming units (CFU) of bacteria was enumerated on agar plates containing appropriate media following serial dilution technique and pour plate method. The media used were yeast extract mannitol agar media (YEMA) for symbiotic N fixing bacteria (Vincent, 1970) and Pikovskaia's agar medium for P solubilizing bacteria (Pikovskaia, 1948).

2.5 DEHYDROGENASE ACTIVITY (Thalmann, 1968)

The method is based on the estimation of the triphenyl tetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) during composting after incubation at 30°C for 24 h. All procedures were performed under diffused light because of the light sensitivity of TTC and TPF. The concentration of TPF (µg ml^{-1}) was calculated after correction for the control value from sample value and the dehydrogenase activity was determined by the following formula:

$$\text{Dehydrogenase activity } (\mu g \text{ TPF } g^{-1}\text{dwt}) = \frac{\text{TPF } (\mu g \text{ ml}^{-1}) \times 50}{\text{dwt} \times W}$$

Where, TPF (µg ml^{-1}) = found from standard curve;
dwt = Dry weight of 1 g soil;
W = Weight of the moist soil;
50 = Total volume of the solution added to the soil.

2.6 PHOSPHATASE ACTIVITY (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977)

The method is based on the determination of *p*-nitrophenol released after the incubation of the soil with *p*-nitrophenol phosphate for 1 h at 37°C using modified universal buffer, MUB (pH 6.5 for the assay of acid phosphatase, pH 11.0 for the assay of alkaline phosphatase) as substrate. The concentration of *p*-nitrophenol (µg g^{-1} dwt h^{-1}) was calculated after correction for the control value from sample value and the phosphatase activity was determined with the following formula:

$$\text{Acid or alkaline phosphatase activity } (\mu g \text{ p-nitrophenol } g^{-1}\text{dwt } h^{-1}) = \frac{C \times V}{Dwt \times Cw \times t}$$

Where, C = Measured concentration of *p*-nitrophenol (µg ml^{-1} filtrate) after correcting the result for the control.
V = Total volume of the suspension (ml);
Dwt = Dry weight of 1 g moist soil;
Cw = Weight of the soil sample used (g);
t = Incubation time (h).

2.7 PHOSPHORUS SOLUBILIZING POWER

Phosphate solubilizing capacities of the soil samples were determined by incubating 1 g soil of each sample in 15 ml Pikovskaia's broth (Pikovskaia, 1948) in culture tubes at 30 ± 1 °C for 15 days followed by estimation of soluble phosphorus in the broth.

2.8 STATISTICAL ANALYSIS

The recorded data were analyzed statistically following the standard procedure as described by Gomez and Gomez (1984). Correlation and regression coefficients of various parameters were calculated. The means were compared using least significant difference (LSD) test, with a significance level of $P \leq 0.05$. Analyses of variance (two way factorial ANOVA) were carried out using a two factor randomized complete block design (fertilization levels x plant growth stages).

3. RESULTS AND DISCUSSION

3.1 BACTERIAL POPULATION

Irrespective of treatment variations, the population of symbiotic N fixing bacteria and P solubilizing bacteria in the peanut rhizosphere significantly ($P \leq 0.05$) increased from 25 DAS and thereafter decreased up to maturity (Table 2). The population of both symbiotic N fixing and P solubilizing bacteria increased significantly with increasing levels of CF under both lime and no lime applied plots. Integrated application of FYM along with CF significantly ($P \leq 0.05$) promoted the bacterial population and augmenting the level of FYM+CF from FYM @ 25 kg to 50 kg N ha^{-1} (i. e., from F_3 to F_4) significantly promoted bacterial proliferation (Table 2). The carbon and nitrogen in FYM+CF could be easily used as energy and nutrient source for soil microorganisms and this resulted in increased soil microbial populations in the rhizosphere soil. This also pointed out that degraded products of FYM was stimulatory towards the growth and proliferation of N fixing and P solubilizing bacteria during the course of decomposition in the soil. Positive influence of compost+CF over sole CF on the population of P solubilizing and N transforming microorganisms was also reported by Limtong & Piriyaprin (2006) and Basu et al. (2011).

The lime-based treatments significantly ($P \leq 0.05$) improved the bacterial population. Rise in soil pH towards neutral due to liming probably promoted bacterial proliferation in the rhizosphere soil. Greater proliferation of *Bradyrhizobium* population with increasing pH towards neutral was also reported by Fettel et al. (1998) and Basu et al. (2011). Barroti and Nahas (2000) stated increase in population of P solubilizing bacteria due to liming.

3.2 ENZYME ACTIVITY

Different levels of CF alone or supplemented by FYM in combination with and without lime significantly influenced the activity of dehydrogenase (DH), acid phosphatase (ACP) and alkaline phosphatase (ALP) enzymes in the rhizosphere soil of peanut (Figure 1) at different growth stages in both the years. Irrespective of treatment variation DH activity was maximum at 25 DAS and thereafter decreased at a slower rate up to 50 DAS, thereafter significantly declined at a faster rate till 75 DAS and again steadily up to 100 DAS, whereas activity of ACP and ALP significantly increased from 25 to 75 DAS and then significantly decreased steadily up to 75 DAS and at a faster rate till 100 DAS.

Increasing the CF level from 0:0:0 (F_0) to 50:25:25 (F_1) kg NPK ha^{-1} significantly improved the activity of DH, however, further increment in CF level to 100:50:50 (F_2) kg NPK ha^{-1} resulted lowering its activity. Decrease in DH activity at maximum level of CF suggests that DH activity was highly sensitive to the inhibitory effects associated with bulky fertilizer additions (Simek et al., 1999).

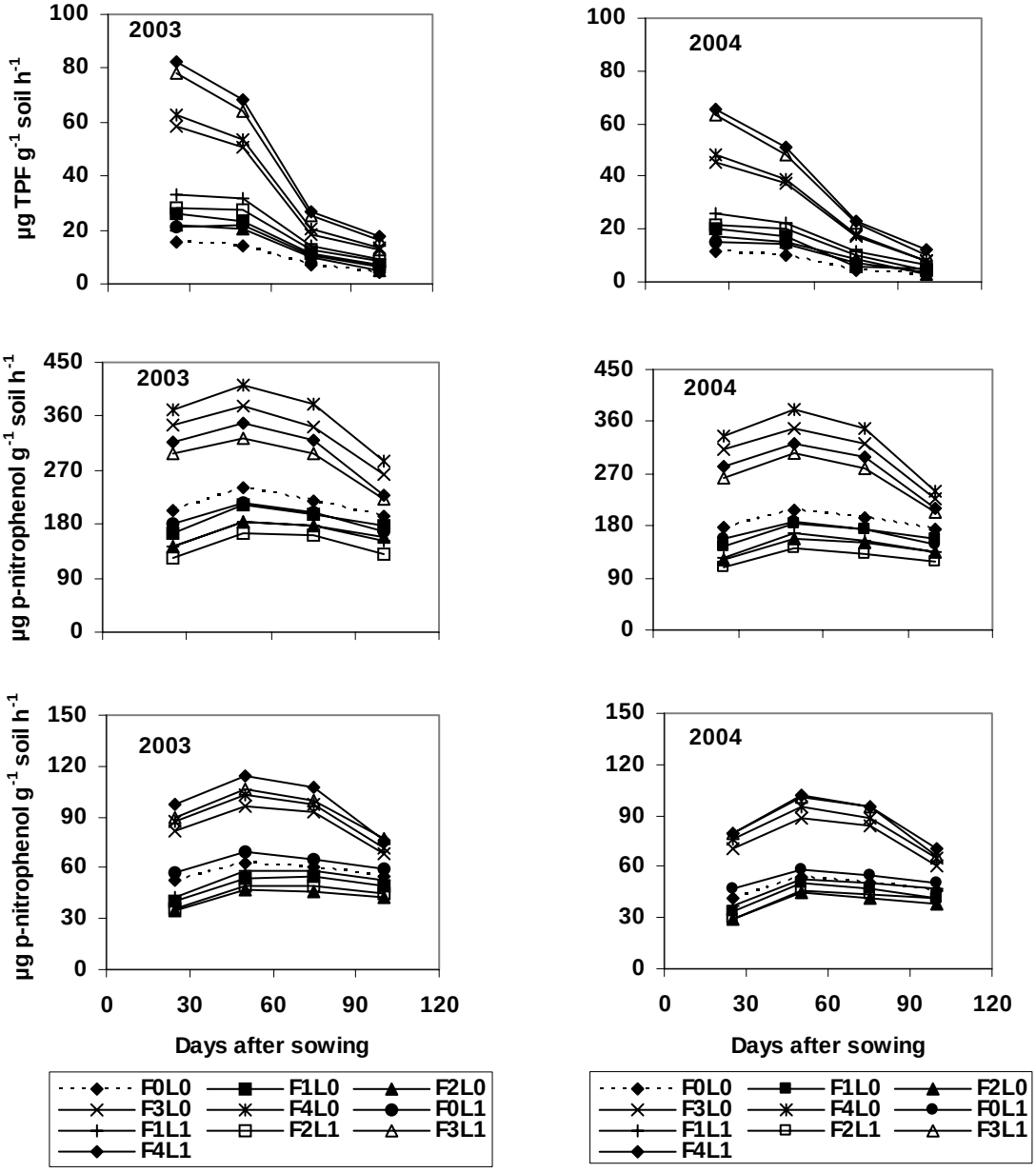
Table 2. Population of bacteria in the rhizosphere soil of peanut at different growth stages as influenced by different fertilization levels during 2003 and 2004

Fertilization levels (F)	Population (Log CFU g^{-1} soil)			
	Symbiotic N fixing bacteria		P solubilizing bacteria	
	2003	2004	2003	2004
F_0	5.23	4.67	4.97	4.49
F_1	5.84	5.12	5.60	4.82
F_2	6.30	5.48	6.05	5.17
F_3	6.73	5.96	6.44	5.75
F_4	7.66	6.57	7.20	6.39
S.E(m)±	**0.122**	**0.086**	**0.099**	**0.115**
LSD (P=0.05)	**0.26**	**0.18**	**0.21**	**0.24**
Lime levels (L)				
No lime	5.99	5.22	5.69	5.03
Lime	6.72	5.90	6.42	5.62
S.E(m)±	**0.077**	**0.054**	**0.063**	**0.073**
LSD (P=0.05)	**0.16**	**0.11**	**0.13**	**0.15**
Growth Stages, Days after sowing				
25	6.32	5.53	6.04	5.24
50	6.74	5.85	6.41	5.68
75	6.54	5.67	6.18	5.43
100	5.81	5.19	5.59	4.95
S.E(m)±	**0.117**	**0.083**	**0.095**	**0.110**
LSD (P=0.05)	**0.23**	**0.16**	**0.19**	**0.22**

F_0 = No chemical fertilizers (CF); F_2 = CF @ 100:50:50 kg ha^{-1} NPK; F_3 = 50% N of F_1 supplemented by farmyard manure (FYM); F_1 = CF @ 50:25:25 kg ha^{-1} NPK; F_4 = 50% N of F_2 supplemented by FYM

The activity of ACP and ALP decreased with increase in the level of CF from 0:0:0 (F_0) to 50:25:25 (F_1) and from 50:25:25 (F_1) to 100:50:50 (F_2) kg NPK ha^{-1}. This was indicative of the fact that there exited a negative relationship between phosphatase activity and inorganic P level. Several studies also showed negative correlations between available P and phosphatase activities (Sarapatka, 2003; Moscatelli et al., 2005). Tadano et al. (1993) stated that P cycle enzyme activities are inversely related to P availability and when P is a limiting nutrient, its demand increases resulting in an increase in phosphatase activity. This phenomenon can be explained by a competitive inhibition of phosphatase by phosphate ions or by a negative-feed back of phosphate ions on PHO genes resulting in a repression of phosphatase synthesis by microorganisms (Oshima et al., 1996).

The lowest level of CF recorded maximum activity of ACP and ALP, although in the earlier section we observed that increasing level of CF caused more proliferation of bacteria in the rhizosphere soil. According to Sarapatka (2003) there is no correlation between phosphatase active colonies of bacteria and ACP and ALP activity in soils. This was probably due to the production of phosphatases by organisms other than bacteria. Such a lack of relationship between microbial density and phosphatase activities has been observed in other studies (Criquet et al., 2004; Ne`ble, 2005; Basu et al., 2011) also.



F_0 = No chemical fertilizers (CF);	F_3 = 50% N of F_1 supplemented by farmyard manure (FYM);	
F_1 = CF @ 50:25:25 kg ha^{-1} NPK;	F_4 = 50% N of F_2 supplemented by FYM;	
F_2 = CF @ 100:50:50 kg ha^{-1} NPK;	L_0 =No lime;	L_1 = Lime @ 2 t ha^{-1}

Fig. 1. Soil enzyme activity in the rhizosphere of peanut at different growth stages as influenced by different fertilization levels during 2003 and 2004

However, the activity of all three enzymes was significantly ($P \leq 0.05$) higher under FYM+CF treated plot as compared to CF alone. Application of FYM @ 50 N kg ha^{-1} (F_4) recorded higher activity of these enzymes as compared to FYM application @ 25 N kg ha^{-1} (F_3). The organic material is the rich source of organic carbon which activated the enzymes like DH, ACP and ALP. A clear positive relationship between soil DH activity and soil C was also reported by Leirós et al. (2000). This result is in agreement with Lee et al. (2004), who reported that soil treated with vermicompost and manure showed higher level of DH activity as compared to mineral fertilizers applied soil. The increase in ACP and ALP activity after additions of FYM along with CF to soils had been generally attributed to the fact that enzyme activities directly associated to organic matter and to microbial response to soluble sugars of the added materials (Nannipieri et al., 1983). Hojati and Nourbakhsh (2006) indicated significant increase in enzyme activities, including phosphatases, due to the addition of CF and organic manure over only CF or control, and thereby corroborated our results.

Application of lime along with CF alone or in combination with FYM+CF significantly ($P \leq 0.05$) enhanced the activity of DH enzyme and such increment was to the extent of 36% as compared to similar combinations but without lime. The enhanced DH activity after lime addition was probably due to the increase in microbial population at raised soil pH as reported by Ray (1985) and Basu et al. (2011). The lime application with any combination significantly decreased the activity of ACP over no lime application, whereas the activity of ALP was improved under lime treated plots. The reason might be due to rise of soil pH to nearly neutral (6.54, data not shown) after soil amelioration, since in acidic soils the acid phosphomonoesterase activity dominates whereas in neutral and alkaline soils the alkaline phosphomonoesterase activity dominates (George et al., 2002; Basu et al., 2011).

3.3 NITROGEN ACCUMULATION IN NODULES AND PHOSPHORUS SOLUBILIZING POWER

It was revealed from results presented in Table 3 that the accumulation of N in nodules and the amount of insoluble P solubilized g^{-1} of rhizosphere soil of peanut at different growth stages were significantly influenced by different fertilization levels during both the years. These values were significantly higher under all fertilization treatments as compared to no fertilizer application under both lime and no lime treatment. The increment of CF level from 50:25:25 to 100:50:50 kg NPK ha^{-1} significantly decreased the content of N in nodules and amount of solubilized P under both lime and no-lime applied plots. These results corroborated the facts that nodule formation and symbiotic N fixation could be reduced by mineral N, while small 'starter' doses of applied N may stimulate nodule formation (Hartfield et al., 1974).

Low P solubilizing capacity might be attributed to less enzymatic activity under higher level of CF application, since only phosphatase enzymes produced by plants and/or microorganisms are able to hydrolyze organic P into phosphates through hydrolysis of both esters and anhydrides of H_3PO_4 (Criquet et al., 2007). Among these enzymes, acid and alkaline phosphomonoesterases and phosphodiesterases are considered as the predominant phosphatases in most types of soil and litter (Criquet et al., 2004).

Application of CF supplemented by FYM @ 25 or 50 kg N ha^{-1} significantly increased the content of N in nodules and P solubilizing capacity as compared to CF alone and doubling-up the level of FYM+CF improved these biological activities. These biochemical activities were significantly increased when lime was applied along with CF alone or FYM+CF.

Table 3. Nitrogen accumulation in nodules and phosphorus solubilizing power of the peanut rhizosphere soil at 4 growth stages as influenced by different fertilization levels during 2003 and 2004

Fertilization levels (F)	N accumulation in nodules (mg N g^{-1} nodules)		P solubilizing power (mg P solubilized 15 mg^{-1} insoluble P g^{-1} soil)	
	2003	2004	2003	2004
F_0	52.3	48.0	0.215	0.179
F_1	64.8	60.2	0.259	0.233
F_2	54.5	49.8	0.224	0.195
F_3	73.2	68.5	0.293	0.267
F_4	74.1	70.0	0.307	0.280
S.E(m)±	1.35	0.98	0.0082	0.0122
LSD (P=0.05)	2.8	2.0	0.017	0.026
Lime levels (L)				
No lime	59.3	54.9	0.239	0.213
Lime	68.3	63.7	0.280	0.249
S.E(m)±	0.85	0.62	0.0052	0.0077
LSD (P=0.05)	1.8	1.3	0.011	0.016
Growth Stages, Days after sowing				
25	61.0	56.7	0.244	0.222
50	70.5	65.4	0.288	0.261
75	66.2	60.8	0.281	0.246
100	57.5	54.3	0.226	0.195
S.E(m)±	1.29	0.93	0.0078	0.0117
LSD (P=0.05)	2.5	1.8	0.015	0.023

F_0 = No chemical fertilizers (CF); F_2 = CF @ 100:50:50 kg ha^{-1} NPK; F_3 = 50% N of F_1 supplemented by farmyard manure (FYM); F_1 = CF @ 50:25:25 kg ha^{-1} NPK; F_4 = 50% N of F_2 supplemented by FYM

Improved N fixation by peanut due to liming was also reported by Raychaudhury et al. (2003) and this might be attributed to the fact that nodules on the lower part of the root system are able to fix more N than crown nodules throughout the growing season and they may contribute most of the N fixed by the legume plant (Hardarson et al., 1989). Liming increased the root length because of increase in exchangeable Ca^{2+} and Mg^{2+} levels in the soil and improved the soil structure providing better aeration in the rhizosphere of peanut (Raychaudhury, 2003). Besides, increased levels of accumulated enzymes in the soil matrix, more importantly, direct contribution of enzymes by the organic manures themselves might also be responsible for greater soil enzyme activity (Dinesh et al., 2000). Higher enzyme activity was responsible for enhanced P solubilizing power of the rhizosphere soil.

Increased bacterial population and enzyme activity under integrated application of FYM, lime and CF was responsible for improved N fixation in nodules and P solubilizing power of the rhizosphere soil throughout the growing season and thereby enhanced the nutrient uptake leading to maximizing dry matter production of peanut (Basu et al., 2011). A positive and significant correlation among bacterial population and enzyme activity vs. N content in nodules and P solubilizing power supported these findings (Table 4).

Table 4. Correlation and regression among different soil biological parameters of peanut rhizosphere as influenced by different fertilization levels during 2003 and 2004

Parameters	2003	2004
Dehydrogenase activity, µg TPF g^{-1} soil h^{-1} (y)		
Population of symbiotic N fixing bacteria,	r = 0.63**	r = 0.65**
Log no. CFU g^{-1} soil (x)	y = 13.40x - 58.51	y = 13.75x - 56.35
Population of P solubilizing bacteria,	r = 0.64**	r = 0.65**
Log no. CFU g^{-1} soil (x)	y = 14.85x - 63.27	y = 13.53x - 51.97
Acid and Alkaline phosphatase activity, µg *p*-nitrophenol g^{-1} soil h^{-1} (y)		
	ACP: r = 0.57**	r = 0.67**
Population of P solubilizing bacteria,	y = 50.92x - 73.55	y = 64.15x - 131.41
Log no. CFU g^{-1} soil (x)	ALP: r = 0.70**	r = 0.79**
	y = 17.32x - 37.13	y = 20.76x - 50.73
N accumulation in nodules, mg N g^{-1} of nodules (y)		
Population of symbiotic N fixing bacteria,	r = 0.84**	r = 0.86**
Log no. CFU g^{-1} soil (x)	y = 9.82x + 1.45	y = 12.08x - 7.86
Dehydrogenase activity, µg TPF g^{-1} soil h^{-1} (x)	r = 0.62**	r = 0.61**
	y = 0.33x + 54.89	y = 0.40x + 51.22
P solubilizing power, mg P solubilized per 15 mg insoluble P g^{-1} soil (y)		
Population of P solubilizing bacteria,	r = 0.85**	r = 0.87**
Log no. CFU g^{-1} soil (x)	y = 0.047x - 0.024	y = 0.06x - 0.06
Dehydrogenase activity, µg TPF g^{-1} soil h^{-1} (x)	r = 0.61**	r = 0.66**
	y = 0.002x + 0.221	y = 0.002x + 0.191
Acid and alkaline phosphatase activity,	ACP: r = 0.65**	r = 0.69**
µg *p*-nitrophenol g^{-1} soil h^{-1} (x)	y = 0.0004x + 0.1658	y = 0.001x + 0.136
	ALP: r = 0.80**	r = 0.82**
	y = 0.002x + 0.139	y = 0.002x + 0.113

*** Significant at 1% level*

3.4 PLANT GROWTH

The growth parameters of peanut like leaf area index (LAI), number of nodules plant^{-1} at 50 DAS and dry matter accumulation (g m^{-2}) at different growth stages were significantly influenced by different levels of CF over no CF application in both the years (Table 5; Figure 2). However, increasing the CF dose from 50:25:25 (F_1) to 100:50:50 kg NPK ha^{-1} (F_2) resulted marginal increment in LAI and dry matter production. According to Satapathy et al. (2005), nitrogen fertilization at optimum level is helpful for the development of the source system and also increases its efficiency for enhanced photosynthesis leading to higher dry matter production. As regards nodule number, the maximum level of CF decreased the nodule formation over 50:25:25 kg NPK ha^{-1} (F_1). Decreased nodule numbers with increasing nitrogen level might be due to lower nodulation in peanut plants (Huang, 1991; Biswas et al., 2003). The LAI under the treatment comprising CF and FYM was comparable with CF except F_1 and F_3 under lime treated plot in the first year, however, in the second year FYM+CF application (F_3 and F_4) resulted significantly higher LAI as compared to CF alone for both lime and no lime application. Similar, favorable effects of different organic manure along with CF on crop performance have been reported (Patel and Patel, 1996; Basu et al., 2006a, 2006b, 2011).

Table 5. Growth parameters at 50 days after sowing of peanut as influenced by different fertilization levels during 2003 and 2004

Fertilization levels (F)	Leaf area index (LAI)			Nodules (Number plant^{-1})		
	Lime levels (L)					
	2003					
	No lime	Lime	Mean	No lime	Lime	Mean
F_0	2.24	2.65	2.45	36.5	46.7	41.6
F_1	3.48	3.92	3.70	58.2	71.8	65.0
F_2	3.62	4.10	3.86	43.7	51.8	47.7
F_3	3.79	4.31	4.05	85.1	103.9	94.5
F_4	3.90	4.42	4.16	59.2	72.4	65.8
Mean	3.41	3.88		56.5	69.3	
	F	L	F x L	F	L	F x L
S.E(m)±	0.172	0.109	0.243	3.07	1.94	4.34
LSD (P=0.05)	0.36	0.23	0.51	6.4	4.1	9.1
	2004					
F_0	1.99	2.27	2.13	31.6	38.9	35.2
F_1	3.07	3.35	3.21	48.4	57.8	53.1
F_2	3.13	3.41	3.27	32.7	39.4	36.0
F_3	3.38	3.82	3.60	67.4	83.8	75.6
F_4	3.49	3.88	3.68	42.6	52.8	47.7
Mean	3.01	3.35		44.5	54.5	
	F	L	F x L	F	L	F x L
S.E(m)±	0.055	0.035	0.078	3.46	2.19	4.89
LSD (P=0.05)	0.12	0.07	0.16	7.3	4.6	10.3

F_0 = No chemical fertilizers (CF); F_2 = CF @ 100:50:50 kg ha^{-1} NPK; F_3 = 50% N of F_1 supplemented by farmyard manure (FYM); F_1 = CF @ 50:25:25 kg ha^{-1} NPK; F_4 = 50% N of F_2 supplemented by FYM

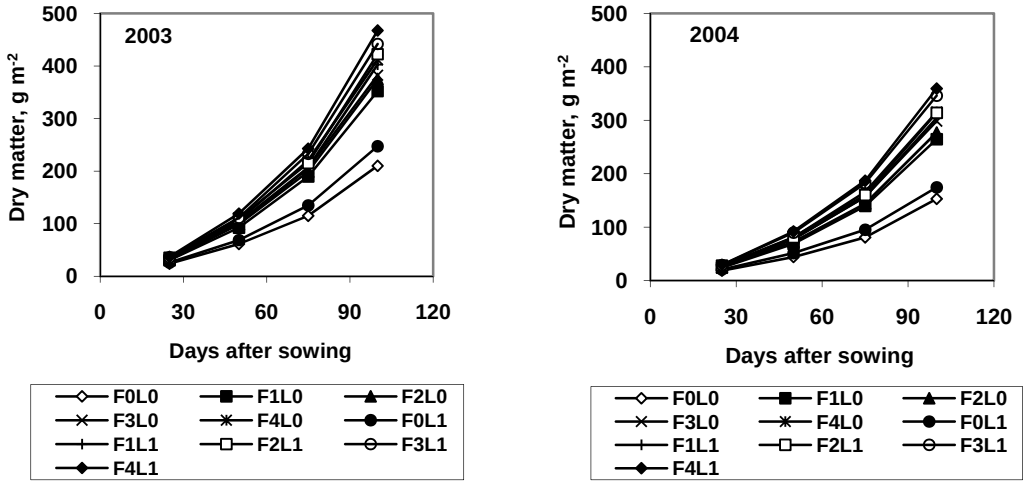


Fig. 2: Dry matter production of peanut at different growth stages as influenced by different fertilization levels during 2003 and 2004

F_0 = No chemical fertilizers (CF); F_2 = CF @ 100:50:50 kg ha^{-1} NPK; F_3 = 50% N of F_1 supplemented by farmyard manure (FYM); F_1 = CF @ 50:25:25 kg ha^{-1} NPK; F_4 = 50% N of F_2 supplemented by FYM

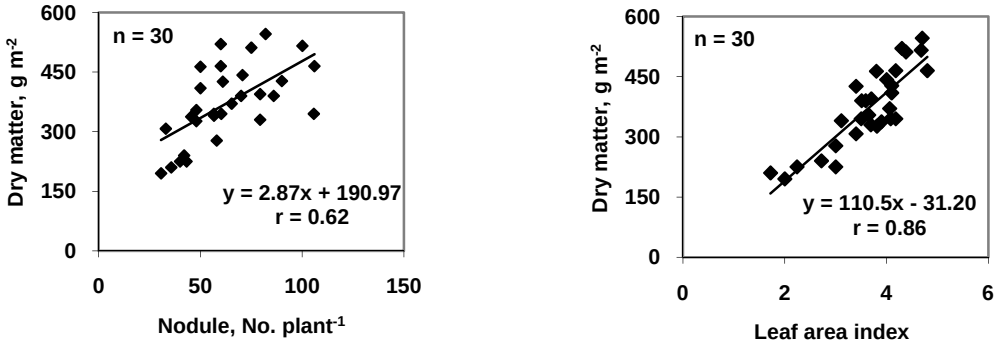


Fig. 3. Correlation and regression between dry matter production vs. number of nodules plant^{-1} and leaf area index at 50 days after sowing of peanut as influenced by different fertilization levels during 2003

The plants in lime treated plots recorded significantly higher LAI, nodule numbers and dry matter as compared to no lime application. Improved nodulation might be due to extended root length and lateral root distribution of peanut in the lime applied plots (Raychaudhury et al., 2003). Two fundamental factors limit the fertility of acid soils viz., deficiency of P, Ca and Mg, and the presence of phytotoxic substances like soluble Al and Mn (Haynes and Mokolobate, 2001).

Liming minimized the stress caused due to either excess of H, Mn, Fe and Al and deficiencies of Ca and Mg, which was helpful in promoting growth and dry matter production of peanut. This is interesting to note that quantity of lime required to raise soil pH beyond 6 for the availability of a large number of plant nutrients depends upon the initial pH of the soil

(Donahue et al., 1990). In the present investigation the level of lime @ 2 t ha^{-1} was decided on the basis of initial soil pH of 5.21 which was to be raised to 6.54 (data not shown). Beneficial effect of liming on the growth and yield of different crops including rice and peanut has been adequately investigated by earlier workers (Raychaudhury, 2003; Basu et al., 2011).

Regarding interaction effect, combined application of FYM+CF along with lime significantly improved all the growth parameters as compared to FYM+CF or sole application of CF. Superior growth parameters resulted in higher dry matter production. This is also clear from a significant and positive correlation between growth parameters and dry matter production of peanut as presented in Figure 3.

It is clear from the results that the performance of peanut in the second year was poorer over first year. With increasing age sabai grass produced more tillers and the leaf length increased (data not shown). As a result it created shade on peanut leading to more than 30% reduction in dry matter production in the second year over first year. In our investigation it was observed that in the first year more than 98% of the full sunlight was available on the top layer of peanut, while it was decreased to 60-65% in the second year. A taller height gives grass an advantage and leads to shading of the legume (Haynes, 1980; Mahapatra, 2011). Legumes generally absorb most of the light they intercept within a few layers of leaves, while grasses are able to distribute and absorb light more evenly throughout the canopy. Therefore, legumes are more susceptible to being shaded by other plants and are poor competitors for light (Guay et al., 2001; Mahapatra, 2011).

4. CONCLUSIONS

Results of present investigation revealed that two factors caused significant effect on soil biological properties: fertilization levels and crop growth stages. Effect of fertilization levels showed that super-optimal level of inorganic fertilizers lowered the enzymatic activity, N fixation in nodules and P solubilization in the rhizosphere soil of peanut, although it exerted positive influence on bacterial proliferation. Integrated application of chemical fertilizer, farmyard manure and lime was more efficient to improve the biological properties (except acid phosphatase activity) in the rhizosphere soil of peanut and thereby improved its growth as compared to chemical fertilizer alone at all the growth stages. Therefore it indicated the fact that microbial densities could not be used as the consistent index for evaluation of soil biological quality. It could also be inferred that soil microbial density, enzyme activity and other microbiological activities were sensitive to even short term organic manuring, which could influence plant growth.

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