



Impact of Long Term Fertilization on Soil Phosphorous Availability in the Rhizosphere of Rice Grown in Acid Inceptisols

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The chemical and biological processes in the rhizosphere not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient-use efficiency of crops and thus profoundly influence crop productivity. Thus, an attempt was made to investigate the relative effects of rhizosphere and non - rhizosphere on the soil phosphorous availability in a rice-rice cropping system during post *kharif* - 2015 in flooded tropical rice (*Oryza sativa* L.) under long term fertilizer experiment (LTFE) started in the year 2005-06 in the central farm of Orissa University of Agriculture and Technology (OUAT) under All India Co-ordinated Research Project (AICRP) in an acidic sandy soil. The experiment was laid out in a randomized block design with quadruplicated treatments. Soil samples at different growth stages of rice were collected from LTFE. The studied long-term manured treatments included 100% N, 100% NP, 100% NPK, 150% NPK and 100% NPK+FYM (5 t ha⁻¹) and an un-manured control. The pH was lower in rhizosphere soil as compared to non- rhizosphere soil in all the three stages of crop growth. Soil fertility status parameters like soil organic carbon (SOC) content and available - P content and microbial biomass phosphorous (MBP) decreased continuously during the crop growth period. Among the fertilized treatments, 100% NPK + FYM exhibited highest nutrient content at maximum tillering stage in both the soils, being recorded highest in rhizosphere soil. The enhanced SOC in rhizosphere soil coupled with high MBP resulted in increased rate of soil - P solubilization. Long term application of balanced and integrated use of fertilizers and organic manure is desirable in order to improve nutrient availability in soil and crop yield. Rhizosphere played a positive role in the nutritional ecology of rice- rice cropping system.

(**Key words:** Long-term fertilization, Phosphorous, Rhizosphere, Rice)

Rice (*Oryza sativa* L.) is one of the most important cereal crops in India, and its production is limited by soil phosphorus (P) deficiency in many parts of the country. Impacts of long-term fertilization regimes on rice productivity and soil-P availability is largely unknown. Soil fertility and P-use efficiency depend, in part, on the complex interactions of the biological, chemical and physical properties of soils and the processes that take place in them.

A better understanding and manipulation of rhizosphere processes may provide an effective approach for improving nutrient use efficiency and crop productivity simultaneously through exploitation of biological potential for efficient acquisition and utilization of nutrients by crops and reducing the over reliance on increased soluble nutrients from application of chemical fertilizers. The chemical and

biological processes that occur in rhizosphere not only determines the mobilization and acquisition of soil nutrients as well as microbial dynamics but also control nutrient use efficiency by crops and thus profoundly influence crop productivity and sustainability (Zhang *et al.*, 2002 and 2004). For these reasons, the management of rhizosphere ecosystem and rhizosphere processes towards sustainable development of the plant-root system may be one of the most important approaches to enhance the utilization efficiency of nutrient resources and crop productivity in rice-based cropping system (Shen *et al.*, 2009).

Most of the acidic soils in India are P deficient, and P fertilizer application is a common practice for overcoming the P deficiency, which causes extreme environmental problems, *e.g.*, soil acidification, soil hardening and P leaching from the soil, ultimately

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reducing the soil fertility (Xiao *et al.*, 2013). To overcome these complications, Xu (2009) investigated the effects of the integrated use of organic fertilizers combined with different inorganic amendments on soil fertility and crop yield. The paddy soils undergo long-term waterlogged conditions that reduce the soil redox potential and change the P sorption and transformation (Liu *et al.*, 2006; Kogel-Knabner *et al.*, 2010). Phosphorus being slowly soluble and mobile in soil, can be rapidly consumed in the rhizosphere by plant uptake, affecting phosphorus concentration gradient in a radial direction away from the root surface. Phosphorus dynamics in the rhizosphere is mainly influenced by the root growth and activity, and also is highly affected by the physicochemical properties of soil. Flooded rice ecosystems present a unique microbial ecology. Soil microbial biomass P, although is very small on an ecosystem basis, plays a central role in understanding changes of P cycling (Coleman and Crosslen, 1996). Thus knowledge of rhizosphere chemistry and rhizosphere process is essential for characterizing nutrient availability in soils. Therefore, the present investigation was undertaken with the principal objective of evaluating the impact of long-term application of mineral fertilizers and farm yard manure and physiological stages of rice growth on soil phosphorous content, soil microbial activities and their relationships with crop yields in humid tropical India.

MATERIALS AND METHODS

The long-term fertilizer experiment of All India Coordinated Research Project (AICRP) of ICAR at OUAT, Bhubaneswar, India (20° 17' N, 85° 49' E and 30 m above mean sea level) since 2005-06 was selected for this study. The location of the experimental site is characterized as sub humid subtropical climate with dry season from October to June and wet season from July to September. The soil of the experimental site was sandy loam in texture, acidic (pH 5.8) and Udic Ustochrept type. Rice cultivar Swarna (MTU-7029) was grown under flooded condition in *kharif* season and Lalat (IET-9947) in *rabi* of every year. Six treatments *viz.*, 100% NPK, 150% NPK, 100% NPK+FYM, 100% N, 100% NP and Control at 80:40:40 kg ha⁻¹ of N:P₂O₅:K₂O in the form of DAP, urea and MOP; 150% NPK; 100% NPK+FYM; FYM at 5 t ha⁻¹ and an unfertilized control were evaluated for the study. Nitrogen was applied in three splits *i.e.* 25% as basal, 50% at 18 days after

transplanting and 25% at panicle initiation stage. Total P was applied as basal and K was applied 50% as basal and 50% at panicle initiation stage. Rice plants (21 days old seedlings of cv.MTU-7029, Swarna) were transplanted @ 2-3 plants hill⁻¹ at a spacing of 15×10 cm² in third week of July. All the field plots remained continuously flooded to a water depth of 3 cm during the entire period of crop growth and were drained 10 days before harvest. Necessary intercultural, water management and plant protection measures were undertaken in general until the crop was matured for harvesting. The experiment was laid out in randomized block design (RBD) and replicated 4 times out of which 3 replications are taken for laboratory analysis.

In this study, soil samples were collected in surface layer (0-15 cm) as well as from rhizosphere zone in each plot at three rice growth stages *viz.*, maximum tillering stage (24 days after transplanting), panicle initiation stage (52 days after transplanting) and maturity stage (100 days after transplanting) in *kharif* season during 2015. For sampling of rhizosphere soil, ten rice plants with root-soil system were randomly dug with intact root system with the help of shovel and shaken up in sterile plastic bags and carried to the laboratory. Five representative non-rhizosphere soil samples (in between plant rows) from each replicated plot were collected randomly from 0-15 cm depth with the help of sample tube and then mixed to make a composite sample.

After sampling, excessive water was drained off on the ground. Visible root fragments and stones were removed manually. After hand crushing, field moist soil samples were stored at 4°C and were used afresh for estimation of soil microbial biomass carbon and microbial biomass phosphorus. A second set of soil samples were air dried and passed through a 2 mm sieve for determination of soil physicochemical and chemical properties. Another triplicate soil samples were used for soil moisture content. Soil moisture content of individual sample was determined gravimetrically in 10 g portion after drying at 105°C for 24 hours.

Soil pH was determined with a glass electrode pH meter (Jackson, 1973). The SOC was determined by dichromate oxidation (Walkley and Black, 1934). The soil available phosphorus was extracted with 0.5M NaHCO₃ solution (pH adjusted to 8.5) and estimated by spectrophotometer as described by Olsen *et al.* 1954. The MBP was determined by fumigation extraction

method (Brookes *et al.*, 1982). All the data were subjected to statistical analysis with software SPSS for significant differences between treatments using analysis of variance (ANOVA) at 5% significance level and correlation between microbial properties and soil available nutrient contents were worked out.

RESULTS AND DISCUSSION

Effect of long term manuring on crop yield and harvest index (HI)

The results of grain yield and harvest index of rice is presented in Table 1. Perusal of the results revealed that there was a significant variation among the different treatments. However, the yield of rice and harvest index have been found to be increased due to different fertilization, being recorded highest yield of rice (5.16 t ha⁻¹) and harvest index (0.48) in the 100% NPK+FYM treatment). Long term application of FYM improves soil fertility including SOC storage and micro and macro nutrients and physical properties (Brar *et al.*, 2015). In contrast, application of balanced fertilization increase crop yield by directly supplying plant nutrients required for crop growth (Ren *et al.*, 2014). The results of the present investigation also find support from the results of the long term experiments reported by Fan *et al.* (2008), Zhang *et al.* (2009) and Mandal *et al.* (2018). The higher or lower amounts of root exudates inputs into soil was associated with higher or lower amount of rice yield under different treatments (King *et al.*, 2001). Among various treatments analyzed, NPK+FYM and NPK produced more straw yields giving higher harvest index. Super optimal dose of NPK *i.e.* 150% produced higher yield than balanced fertilization *i.e.* 100% NPK (4.18 t ha⁻¹) but lower straw yield cause lower harvest index. Unfertilized control plot showed

significantly lowest yield (2.20 t ha⁻¹). Among the fertilizer treatments, the lowest yield (3.55 t ha⁻¹) was recorded in the treatments receiving only N *i.e.* 100% N. But application of nitrogen alone @ 80 kg ha⁻¹ increased yield by 61.73% over unfertilized control treatment. Application of phosphorus resulted in 15.21% of more yield over 100% N treatment.

Effect of long term manuring on soil properties pH

Comparison of means (Table 2) indicated that pH of the rhizosphere soil in all the three stages of crop growth was significantly lower than non-rhizosphere soil. pH of the soil was significantly affected by fertilizer treatments, stages of rice growth and their interaction. After submergence, the pH in both the soils significantly increased up to panicle initiation stage and reached more or less stable value at maturity stage. Application of fertilizers significantly decreased the pH compared to unfertilized control at all growth stages of rice in both the soils. Decline in pH might have resulted from build-up of organic matter with time in fertilizer plots and also use of urea fertilizer and build-up of organic matter might have resulted in decrease in pH (Benbi and Brar, 2009). But 100% NPK+FYM in both rhizosphere and non-rhizosphere soil (5.64, 5.73 respectively) maintained significantly higher level of pH over other fertilized as well as unfertilized control plots. The application of organic manure could improve soil acidity by increasing the soil organic matter, promoting the soil maturation, improving the soil structure, and enhancing the soil base saturation percentage (Li *et al.*, 2010). There was no significant variation between crop growth stage and types of soil and also in between treatment and crop growth stages.

Soil organic carbon (SOC) content

The results given in Table 3 revealed that the magnitude of soil organic carbon (SOC) content was highest in rhizosphere soil as compared to that of non-rhizosphere soil. Mean seasonal SOC content ranged from 6.2 g kg⁻¹ in control to 16.3 g kg⁻¹ in plots receiving balanced fertilization along with organic manure in rhizosphere soil and 4.4 g kg⁻¹ in control to 12.2 g kg⁻¹ in 100% NPK+FYM treatment in rhizosphere soil. The increase in SOC could be due to increased underground biomass through organic matter addition (Zhang *et al.*, 2009). SOC content was 22% higher in

Table 1. Influence of organic amendments and inorganic fertilizers on yield and harvest index of rice under different fertilizer combinations

Treatments	Grain yield (t ha ⁻¹)	Harvest index
100 % NPK	4.18	0.47
150% NPK	4.66	0.44
100 % NPK +FYM	5.16	0.48
100 % N	3.55	0.47
100 % NP	4.09	0.47
Control	2.20	0.44
LSD (P = 0.05)	Grain Yield = 4.83	Harvest Index = 0.029

Table 2. Effect of long term manuring on the changes of pH at different growth stages of rice

Treatment	Crop growth stages (CGS)							
	MT		PI		MA		Grand Mean	
	R	NR	R	NR	R	NR	R	NR
100 % PK	5.17	5.27	5.23	5.32	5.20	5.23	5.20	5.27
100 % NPK	5.20	5.34	5.30	5.40	5.24	5.37	5.25	5.37
150% NPK	5.31	5.40	5.34	5.45	5.30	5.32	5.32	5.39
100 % NPK +FYM	5.60	5.75	5.74	5.83	5.59	5.62	5.64	5.73
100 % N	5.20	5.33	5.23	5.43	5.20	5.36	5.21	5.37
100 % NP	5.23	5.46	5.30	5.53	5.22	5.44	5.25	5.48
Control	5.42	5.44	5.43	5.49	5.38	5.41	5.41	5.45
Mean	5.30	5.43	5.37	5.49	5.30	5.39	5.33	5.44

LSD(P=0.05)

T	Z	CGS	T X Z	T x CGS	Z x CGS	T x Z x CGS
0.035	0.014	0.017	0.05	NS	NS	NS

MT- Maximum tillering, PI- Panicle initiation, MA- Maturity, R- Rhizosphere soil, NR- Non rhizosphere soil, T- treatment, Z- Zone

Table 3. Effect of long term manuring on the changes of soil organic carbon (g kg^{-1}) at different growth stages of rice

Treatment	Crop growth stages (CGS)							
	MT		PI		MA		Grand mean	
	R	NR	R	NR	R	NR	R	NR
100 % PK	7.8	7.4	7.2	5.7	6.2	4.5	7.07	5.87
100 % NPK	9.2	8.2	8.7	6.3	8.0	4.4	8.63	6.30
150% NPK	11.6	9.8	9.8	9.2	9.3	5.4	10.23	8.13
100 % NPK +FYM	17.7	16.8	17.0	13.9	14.2	6.1	16.30	12.27
100 % N	8.4	7.5	5.6	5.3	4.5	4.7	6.17	5.83
100 % NP	8.7	8.3	7.2	7.7	5.5	5.2	7.13	7.07
Control	7.9	4.9	5.8	4.4	4.7	3.9	6.13	4.40
Mean	10.19	8.99	8.76	7.50	7.49	4.89	8.81	7.12

LSD(P=0.05)

T	Z	CGS	T X Z	T x CGS	Z x CGS	T x Z x CGS
0.49	0.20	0.24	0.69	0.85	0.35	1.20

MT- Maximum tillering, PI- Panicle initiation, MA- Maturity, R- Rhizosphere soil, NR- Non rhizosphere soil, T- treatment, Z- Zone

rhizosphere soil compared to non-rhizosphere soil. Fertilization had varying effects on SOC content in both type of soils. It was observed that 100% NPK+FYM was significantly better than rest of the treatments. Imbalanced fertilization did not differ significantly in their SOC content. Super optimal dose of NPK recorded 18% higher SOC in rhizosphere soil and 28% in non-rhizosphere soil over balanced fertilization. During the rice crop growth stages, maximum tillering stage of rice crop found to have highest SOC content (10.19 g kg^{-1})

¹) followed by panicle initiation stage (8.76 g kg^{-1}) and maturity stage (7.49 g kg^{-1}) in rhizosphere soil. Similar trend was found in case of non-rhizosphere soil such as maximum tillering stage (8.99 g kg^{-1}) > panicle initiation stage (7.50 g kg^{-1}) > maturity stage (4.89 g kg^{-1}).

Available phosphorus

Mean seasonal available phosphorus content (Table 4) increased significantly by 25% under rhizosphere soil over non-rhizosphere soil. Mean

Table 4. Effect of long term manuring on the changes of available phosphorus (kg ha^{-1}) at different growth stages of rice

Treatment	Crop growth stages (CGS)							
	MT		PI		MA		Grand mean	
	R	NR	R	NR	R	NR	R	NR
100 % PK	10.70	6.80	8.97	5.38	8.67	5.17	9.45	5.78
100 % NPK	15.20	11.77	14.68	10.83	12.84	9.55	14.24	10.72
150% NPK	24.50	22.00	23.18	22.87	21.00	19.09	22.89	21.32
100 % NPK +FYM	37.10	34.90	46.08	30.28	41.26	27.24	41.48	30.81
100 % N	7.60	6.77	6.37	5.83	5.50	5.32	6.49	5.97
100 % NP	10.43	8.57	8.57	7.49	6.62	6.75	8.54	7.60
Control	6.60	4.20	5.34	4.18	4.83	3.72	5.59	4.03
Mean	16.02	13.57	16.17	12.41	14.39	10.98	15.53	12.32
LSD(P=0.05)								
T	Z	CGS	T X Z	T x CGS	Z x CGS	T x Z x CGS		
1.73	0.71	0.87	2.45	3.01	NS	NS		

MT- Maximum tillering, PI- Panicle initiation, MA- Maturity, R- Rhizosphere soil, NR- Non rhizosphere soil, T- treatment, Z-Zone

seasonal available phosphorus content ranged from 5.59 kg ha^{-1} in control treatment to 41.48 kg ha^{-1} in the plots receiving balanced fertilization along with organic manure *i.e.* 100% NPK+FYM in rhizosphere soil and similarly in non-rhizosphere soil, control plots showed the lowest P content (4.03 kg ha^{-1}) and 100% NPK+FYM treated plot registered highest P content (30.81 kg ha^{-1}). An enhancement of the bioavailability of soil inorganic P as a consequence of rhizosphere acidification also been shown for low land rice growing in a flooded soil by some other researchers (Begg *et al.*, 1994; Kirk and LevanDu, 1997) and also plant root exudates released in the rhizosphere contains phosphorous radicals (Fageria and Stone, 2006). Highest available P was found in FYM treated plots as farmyard manure (FYM) is a good source of phosphorus by releasing organic acids and H^+ that solubilizes inorganic P (Fageria and Stone, 2006). In earlier study Manna *et al.* (2007) had observed the least P fixation was in FYM treatment plots which could be due to blocking of the P fixation sites by organic molecules released from the decomposing manure.

Among the fertilized treatments, 150% NPK showed highest magnitude of available phosphorus content followed by balanced fertilization. Addition of phosphorus in the fertilized treatment increased around 32% and 27% more phosphorus than sole source of nitrogen only in rhizosphere and non-rhizosphere soils respectively. Available phosphorus content in both the soils did not differ significantly in crop growth stages. Available phosphorus content was maximum at maximum tillering stage in all the treatments except the

treatments receiving either combination of organic and inorganic or organic-inorganic combination along with soil amendments or only amendment only in rhizosphere soils. These treatments recorded highest available phosphorus at panicle initiation stage. But in case of non-rhizosphere soil, the available P was maximum at maximum tillering stage and then gradually decreased or was more or less stable up to maturity stage.

Microbial biomass phosphorus (MBP)

It was observed that average soil MBP content (Table 5) under rice-rice cropping systems differed significantly in all stages of growth. The MBP content was in the range of 14.04 to 45.43 , 9.32 to 33.52 , 5.77 to $22.89 \mu\text{g g}^{-1}$ dry soil in rhizosphere soil and 6.64 to 32.69 , 4.65 to 24.62 , 3.39 to $16.43 \mu\text{g g}^{-1}$ dry soil in non-rhizosphere soils at maximum tillering stage, panicle initiation stage and crop maturity stages respectively and the maximum MBP content was recorded in maximum tillering stage of crop development. MBP content gradually decreased throughout the crop growth period which may be due to less root exudation coupled with soil drainage management in later stages of rice crop (Aulakh *et al.*, 2001). There was significant difference in MBP content under rhizosphere and non-rhizosphere soil. MBP content was 42% higher in rhizosphere soil than non-rhizosphere soil as rhizosphere soil is the store house of many mycorrhizae fungi (Fageria and Stone, 2006). Mean seasonal MBP content was lowest *i.e.* $9.71 \mu\text{g g}^{-1}$ dry soil in control plot and highest ($33.94 \mu\text{g g}^{-1}$ dry soil) in 100% NPK+ FYM treated plot under rhizosphere soil. Similarly, lowest MBP ($4.89 \mu\text{g g}^{-1}$ dry

Table 5. Effect of long term manuring on the changes of microbial biomass phosphorus ($\mu\text{g/g}$ of dry soil) at different growth stages of rice

Treatment	Crop growth stages (CGS)							
	MT		PI		MA		Grand mean	
	R	NR	R	NR	R	NR	R	NR
100 % PK	17.77	9.35	10.18	6.57	8.98	5.17	12.31	7.03
100 % NPK	35.20	22.69	24.26	17.90	11.26	10.87	23.57	17.15
150% NPK	36.57	24.31	23.35	18.06	16.18	13.39	25.37	18.59
100 % NPK +FYM	45.43	32.69	33.52	24.62	22.89	16.43	33.95	24.58
100 % N	15.43	13.40	11.61	6.14	6.51	4.95	11.18	8.16
100 % NP	19.29	12.85	16.00	10.27	8.58	5.30	14.62	9.47
Control	14.04	6.64	9.32	4.65	5.77	3.39	9.71	4.89
Mean	26.25	17.42	18.32	12.60	11.45	8.50	18.67	12.84
LSD(P=0.05)								
T	Z	CGS	T X Z	T x CGS	Z x CGS	T x Z x CGS		
1.08	0.44	0.54	1.59	1.87	0.76	2.65		

MT- Maximum tillering, PI- Panicle initiation, MA- Maturity, R- Rhizosphere soil, NR- Non rhizosphere soil, T- treatment, Z-Zone

Table 6. Correlation coefficient (r) among different measured rhizosphere and non rhizosphere soil parameters and yield

	pH	SOC	Av. P	MBP
Rhizosphere soil				
Yield	0.36	0.78**	0.80**	0.88**
pH	1	0.83**	0.80**	0.67*
SOC	0.83**	1	0.99**	0.94**
Av. P	0.80**	0.99**	1	0.95**
MBP	0.67*	0.94**	0.95**	1
Non rhizosphere soil				
Yield	0.50	0.86**	0.84**	0.90**
pH	1	0.80**	0.72**	0.62*
SOC	0.80**	1	0.94**	0.87**
Av. P	0.72**	0.94**	1	0.93**
MBP	0.62*	0.87**	0.93**	1

*, ** marked correlations are significant at $p=0.05$ and 0.1 , respectively.

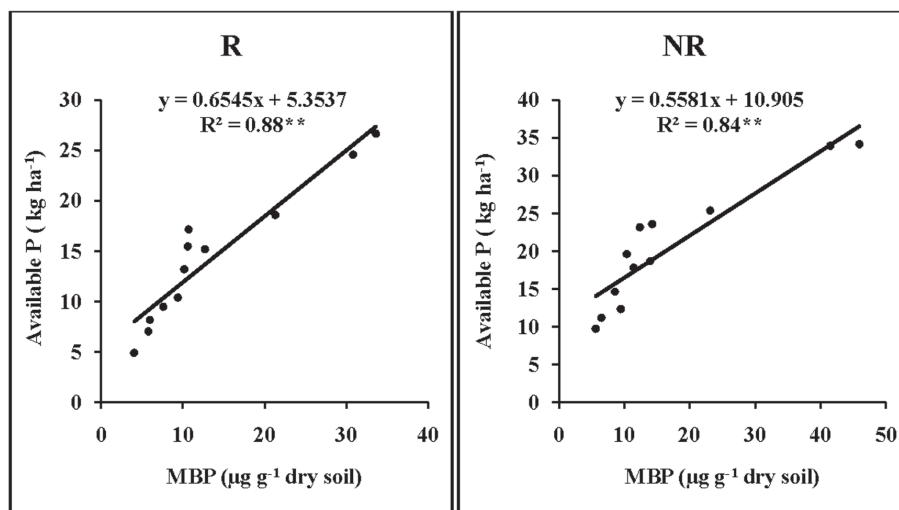


Fig. 1. Relationship of MBP with available P at R and NR soils respectively
 ** next to R^2 values indicate significant a $p<0.01$

soil) in unfertilized control plot and highest MBP (24.58 $\mu\text{g g}^{-1}$ dry soil) in 100% NPK+FYM treated plot were recorded in non-rhizosphere soil. Organic materials like FYM @ 5 t ha^{-1} input organic carbon to soil, stimulated the microbial growth and activity (Kanchikerimath and Singh, 2001).

Relationship among different measured soil parameters and with crop productivity

The coefficient of correlations amongst various parameters showed that all parameters were significantly correlated with each other in both rhizosphere and non-rhizosphere soils (Table 6). The microbial parameters namely MBP were positively and significantly related with SOC content, but it showed highest correlation ($r = 0.94^{**}$) in rhizosphere soil. Similarly, MBP exhibited highest correlation ($r = 0.95^{**}$) with available P in rhizosphere soil compared to ($r = 0.93^{**}$) non-rhizosphere soil and it was positively and more significantly correlated with available P rather than SOC content and pH in both soils. The observed significant correlations among different parameters in the soil indicate the existence of a dynamic equilibrium among them. This means that depletion or enrichment in one would change the other. Such relationship particularly for MBP with soil organic carbon and available phosphorus were stronger. This emphasizes the importance of monitoring these parameters for better understanding of nutrient availability in the soil. Yield showed highest correlation with MPB at both the soil. The available phosphorus content in soil was strongly related with MBP (Fig. 1) and again soil organic carbon content is significantly correlated with MBP (Table 6) in both the soils suggesting soil organic carbon and available phosphorus content in the soils are an important carbon, energy and nutrient source for soil microorganisms.

From the entire study it could be concluded that soil organic matter decomposition and nutrient availability can be enhanced by root soil interactions in the rhizosphere compared to non-rhizosphere soils. Microbial biomass phosphorous has a highly significant positive correlation with SOC and available P in both soils. The enhanced organic carbon in rhizosphere soil coupled with high MBP resulted in increase rate of soil P solubilization. This demonstrates the importance of microbial parameters particularly for nutrient availability in rhizosphere soil and in general rice crop. Maximum tillering stage of rice growth played the

most significant role to enhance nutrient availability. Long term application of balanced and integrated use of fertilizers and organic manure is most desirable in order to improve nutrient availability in soil and crop yield. Overall, rhizosphere played positive role in the nutritional ecology of rice-rice cropping system

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