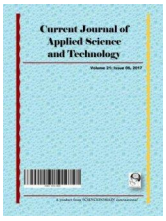

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Correlation of Microbial Population with Enzymatic Activities and Nutrient Levels of Soil during Paddy Growth

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Authors' contributions

This work was carried out in collaboration between all authors. Author JK performed the experiment. Author SKG planned and managed to conduct the study. Author SSW managed to conduct the study in field. All authors read and approved the final manuscript.

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ABSTRACT

The influence of growth stages of paddy on correlation of different microbial population with soil enzymatic activities (dehydrogenase, urease and alkaline phosphatase), available nutrient pool and organic carbon has been accessed. These soil parameters were investigated at four growth stages viz., at transplantation, tillering, reproductive and maturation stage. Results showed that total bacterial and diazotrophic population had a significant positive correlation with soil dehydrogenase, alkaline phosphatase, available NPK and organic carbon at each growth stage. Fungal and actinobacteria population, however; showed a variable trend with various soil parameters at different growth stages of the crop. Actinobacteria population showed significant positive correlation with soil enzymatic activities and available nutrient at the reproductive stage; whereas it showed a negative correlation with most soil parameters at other time intervals. A significant effect of paddy growth stages was recorded on microbial community structure and their activities. At tillering stage, fungal, bacterial and diazotrophic population have significant impact on soil enzymatic activities;

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However, at reproductive stage, actinobacteria population become greater contributor towards soil enzymatic activity as compared to fungal population. This study revealed a complex plant-soil relationship, owing to the role of alternating microbial dynamics in determining soil health and fertility.

Keywords: Available nutrients; correlation; enzymatic activities; microbial population; paddy.

1. INTRODUCTION

The growth of plants in soil affects the biological and physico-chemical properties of the soil environment close to the growing roots [1]. The rhizodeposition of nutrients by plant roots supports increased microbial growth in comparison with that of the bulk soil communities, a phenomenon often referred to as the 'rhizosphere effect' [2]. The interaction between plant nutrients in soil and plant exudates modifies the micro climate of the rhizosphere [3]. Roots secrete organic compounds that are responsible for enhancing microbial population in rhizosphere zone due to increased availability of carbon (C) serving as food and energy [4].

The inseparable plant-microorganism system undergoes short and long term fluctuation depending on plant development stage as well as agro-ecological condition [5]. A plant is a partner in the biocenotic system and all the physiological changes it undergoes during growth are reflected in the feature of coexisting microorganisms. Paddy soil is considered a unique agro-ecosystem as it is kept flooded during the crop growth and is drained during the off crop season. Microbial communities within paddy fields vary in diversity in response to environmental changes. Some of the factors that influence microbial activities in paddy fields are growth stage of the plant, quality as well as quantity of organic materials, seasonal variation, oxygen availability, temperature, moisture status and inorganic fertilisers. Microorganisms in soil regulate the dynamics of organic matter decomposition and plant nutrient availability thus play a key role in the responses of ecosystems to global climate changes [6].

The crops grow in an interacting ecosystem made up of soil-microorganism-plant and atmosphere. So, the objective of the study was to assess the correlation of soil microbial population with enzymatic activities and the available nutrient pool of soil varied over time and if such relation were similar irrespective of the farming type.

2. MATERIALS AND METHODS

The long term field experiment, since 2001 was conducted in the Agronomy Department Research Farm of the Punjab Agricultural University (PAU), Punjab, India. Rhizospheric soil samples were collected from this experiment during *kharif* season of 2013-14. The soil has loamy sand texture with pH 7.80 and EC 0.38. Initial nutrient content of the soil was recorded as 134.4 kg/ha nitrogen, 26 kg/ha phosphorous and 140.0 kg/ha potassium. During paddy cropping season, the mean maximum and minimum temperature ranged between 30.6°C- 44.1°C and 21.0°C- 29.1°C, respectively. Mean rainfall was recorded 476.0mm with relative humidity in range of 72-83 percent. Maximum rainfall was recorded in 24th and 33rd standard meteorological week.

The experiment was laid down in a random block design with three replications. Total five treatments were designed using different combination of inorganic fertilisers, green manure and plant density. *Crotolaria juncea* was used as green manure in the experiment. The whole plant was incorporated into the soil which provide 50-75 kg/ha nitrogen, 15-20 kg P_2O_5 and 40-60 kg K_2O approximately [7]. The inorganic nutrients such as nitrogen (N), phosphorus (P) and potassium (K) were supplied with urea (46% nitrogen), diammonium phosphate (18% N and 46% P) and muriate of potash (60% K), respectively. The recommended dose of inorganic fertilisers (NPK) were applied as per the PAU, package and practices; which is 120 kg/ha nitrogen, 30 kg/ha potassium and 30 kg/ha phosphorous. The nitrogen was applied in three splits i.e., 1/3rd at time of puddling; 1/3rd at three weeks after transplanting and remaining 1/3rd was applied at six weeks after transplanting. The recommended dose of P and K were applied at the time of transplanting. Total of twenty four irrigations were applied during crop growth.

T1: 180 N/ha + 22 plants /m^2 (farmer's practice)
T2: Recommended dose of NPK (120-30-30) kg/ha + 33 plants/m^2
T3: Recommended NPK + Green manure (15 t/ha) +33 plants/m^2

T4: Recommended NPK + Green manure (15 t/ha) +44 plants/m^2
T5: Fertiliser on soil test basis + 33 plants /m^2

Soil samples were collected at four different time intervals during the paddy growth i.e., at transplantation, at tillering, at reproductive and at maturity stage from all treatments. Five soil samples were randomly taken from the field under similar treatment. These soil samples were mixed together to get one representative sample. Soil samples were enumerated for different soil microbial population using serial dilution spread plate technique on their respective culture medium. Appropriate dilutions of the soil samples were plated on: nutrient agar for bacteria, glucose yeast agar for fungi, Kenknight's agar for actinobacteria and Jensen's for diazotrophs incubated at 32°C for 24 hr, 28°C for 24 hr, 32°C for 72 hr and 32°C for 120 hr, respectively (Table 1). The petri plates were observed daily for fast growing microorganisms; colonies with desired traits on different media were counted and recorded as colony-forming units (CFU) $gram^{-1}$ dry soil. Soil samples were also analysed for different enzymatic activities such as dehydrogenase, urease and alkaline phosphatase following standard protocols of Klein et al. [12], Bremmer and Douglas [13] and Tabatabai and Bremner [14], respectively.

A part of representative soil samples was air dried in shade, grinded and sieved to assess soil chemical properties such as available nitrogen, available phosphorous, available potassium and organic carbon following protocols given by Subbiah and Asija [15], Olsen et al. [16] Mervin and Peech [17] and Walkley and Black [18], respectively.

2.1 Statistical Analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) 16 version software [19]. Pearson correlation was used to determine the correlation of microbial population with enzymatic activities and available nutrient pool of the soil.

3. RESULTS AND DISCUSSION

Application of green manure significantly increased the soil enzymatic activities as well as available nutrient pool in soil. Soil enzymatic activities flourished more at flowering stage, owing to the increased amount of root exudates which positively influenced the enzymatic activities and thus enhance availability of nutrient in paddy soil [20]. The increase in plant density (i.e., 22 plants/m^2 to 33 plants/m^2) positively influenced the enzymatic activities and nutrient level in paddy rhizosphere [21]. This might be due to the application of green manures and increased plant density that facilitate an optimal growth and activity of indigenous soil microorganism which consequently affects the available nutrient in soil. To study the link between indigenous microbial population with enzymatic activities and available nutrient pool of the soil, we determined the correlation between these factors at different crop growth stages.

3.1 Correlation of Indigenous Microbial Population and Soil Enzymatic Activities at Different Paddy Growth Stages

Enzymatic activity is the potential index that can fully reflect the changes in the soil biology. Further, soil biology is common index that indicates the soil productive forces and quality [22]. The activity of soil enzymes can be influenced by nature and age of crops, soil microbial population, ecological disturbances, addition of fertilisers and manures. In present study, correlation of microbial population with soil enzymes such as dehydrogenase, urease and alkaline phosphatase was studied.

3.1.1 Indigenous microbial population and soil dehydrogenase

Soil dehydrogenases (EC 1.1.1) are the representatives of the oxido-reductase enzyme class. Among all enzymes in the soil environment, dehydrogenases are one of the most important intracellular enzymes and are used as an indicator of overall soil microbial activity. It was observed that the total bacterial and diazotrophic population; showed highest significant positive correlation with soil dehydrogenase activity at all crop growth stages. This elucidated that bacterial and diazotrophic population remained the most active microbial group during whole crop growth period. Results were supported by the findings of Yuvraj [23] that dehydrogenase had significant positive correlation with total bacterial population at all-time intervals of paddy crop. Alvear et al. [24] had also reported a close relationship between dehydrogenase activity and microbial biomass.

Fungal population showed a non-significant positive relation with soil dehydrogenases at time of transplantation (r=0.469) and reproductive stage (r=0.389) whereas, the inter-relationship

(r=0.812, p=0.01) was found significantly positive at tillering stage. Actinobacteria population showed a variable relation with soil dehydrogenases during different plant growth stages, as its correlation was found significantly negative at time of transplantation (r=-0.720, p=0.01) and at tillering stage (r= -0.759, 0=0.01). This can be explained by negative correlation of actinobacteria population with that of other microbial groups at both these time intervals. On contrary, very high correlation coefficients were recorded actinobacteria (r=0.719, p=0.01) population with soil dehydrogenases at reproductive stage (Table 2). It may be attributed to the increase in the root exudates at reproductive stage as compared to other time intervals. This might be due to the less competition posed by other microbial communities to slow growing actinobacteria at reproductive stage, as indicated by the positive correlation of actinobacteria with bacterial (r=0.849, p=0.01), fungal (r=0.059) and diazotrophic population (r=0.652, p=0.01) at this particular stage. Similar results were reported by Kaur and co-workers [25] that bacterial population and fungal population showed positive correlation with soil dehydrogenase whereas, actinobacteria showed significant negative correlation with soil dehydrogenase. Houlden et al. [26] observed that the bacterial populations had increased with a reduction of the fungal populations, at the reproductive stage of pea.

3.1.2 Indigenous microbial population and soil urease

Soil urease (EC 3.5.1.5) directly participates in soil transformation of the nitrogenous organic compounds and its activity can reflect the nitrogen level in the soil to some extent. The factors that determines overall index of soil urease include microbial community, physical and chemical properties of soil [27]. Present study resulted in bacterial population showing a significant positive correlation with soil urease at time of transplantation(r=0.764, p=0.01) and at tillering stage (r=0.866, p=0.01). This might be due to application of inorganic nitrogen fertilisers (urea) in fields. At these growth stages, bacterial population were found be the active microbial flora which converted inorganic urea to ammonia by utilising urease enzyme. However, the positive but non-significant correlation of bacterial population was observed with urease activity at reproductive (r=0.403) and maturity stage (r=0.355). This could be attributed to absence of direct application of inorganic N fertilisers in soil. Fungal and actinobacteria population were found to have variable relation with soil urease activity during various crop growth stages. As in case, the correlation analysis of fungal population with soil urease (r=0.812, p=0.01) was positive at tillering stage only, which however, was found non-significant at all other growth stages. Actinobacteria population showed a significant negative correlation with soil urease at tillering (r=-0.705, p=0.01) and maturity stage(r=-0.633, p=0.05). This negative relation can be explained by the negative correlation of actinobacterial population with other microbial flora at same time intervals (Table 2). Diazotrophic population showed a significant positive correlation with soil urease during paddy growth period. This may be due to the alternative flooded condition during crop growth. Similar results were observed by Omari et al. [28] that high moisture content increased the *Azotobacter* populations, while *Azospirillum spp.* populations were significantly reduced.

3.1.3 Indigenous microbial population and soil alkaline phosphatase

Alkaline phosphatise (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from various molecules in soil, including nucleotides, proteins, and alkaloids. In Gram-negative bacteria, alkaline phosphatase is located in the periplasmic space, external to the cell membrane, thus were subjected to environmental variation than those actual interior of the cell. This study resulted in significant positive correlation of total bacterial and diazotrophic population with alkaline phosphatase activity during various crop growth stages. This might be due to comparatively resistant nature of bacterial alkaline phosphatase to inactivation, denaturation and degradation and also has a higher rate of activity. Fungal population showed positive correlation with alkaline phosphatase activity at both transplantation(r=0.373) and at tillering stage (r=0.639, p=0.05), whereas showed non-significant negative correlation at other growth stages (Table 2). Alternatively, actinobacteria population showed a significant negative correlation with alkaline phosphatase at transplantation(r=-0.711, p=0.01) and tillering stage (r=-0.859, p=0.01). It showed a significant positive correlation coefficient (r=0.560, p=0.05) only at reproductive stage, whereas, remained negatively correlated with alkaline phosphatase at all other time intervals. Results were in accordance with studies of Gardner et al. [29] that fungal population showed a positive

correlation with phosphatase activity during crop growth stages.

The results showed by Lu Sheng et al. [30] suggested that the soil enzymatic activities were also affected by paddy growth stages as from the tillering to filling stages, the paddy was at the most flourished stage and the soil enzymatic activities were at strongest stage, the paddy roots excreted more organic acid and carbohydrate, which stimulated the correlative soil enzymatic activities.

3.2 Correlation Analysis of Indigenous Microbial Population with Soil Available Nutrients

Soil microorganisms are crucial for nutrient cycling, soil fertility and productivity of every crop. Maintenance of soil microbiota is important for soil fertility and health. In present study, correlation was assessed to determine the effect of indigenous microflora on available nutrient and organic carbon of the soil.

Nitrogen is one of the major nutrients required by plants for their growth. The total nitrogen present in soil is nearly 90-99% of the organic forms and 1-5% of the inorganic form mainly as ammonium and nitrate ion. In this study, bacterial and diazotrophs were the population that remained positively correlated at significance level of 0.01; with soil available nitrogen at all growth stages. Fungal population showed significantly high positive correlation (r =0.922, p=0.01) with available nitrogen at tillering stage. However, the correlation was positive but non-significant with available nitrogen at all other crop growth stages. Actinobacteria population showed negative correlation with available nitrogen at transplantation (r = -0.375) and tillering stage (r=-0.863, p=0.01). However, at reproductive stage interrelationship was found significantly positive (r= 0.712, p=0.01) with available nitrogen (Table 3). This might be due to the high activity of actinobacteria at reproductive stage as compared to other growth periods.

Available phosphorous and potassium showed significant and maximum relation with total bacterial and diazotrophic population with maximum correlation coefficients at each crop growth stage. Available phosphorous showed significantly positive correlation with fungus (r= 0.783, p=0.01) and actinobacteria (r= 0.748, p=0.01) at tillering and reproductive stages, respectively. These results can be explained in terms of positive correlation of these populations with alkaline phosphatase enzyme at respective growth stages. At reproductive and maturity stages, fungus population showed non-significant negative correlation with available phosphorous. Similar relation of microbial population was observed with soil available potassium as those observed with available phosphorous (Table 3). Rezende et al. [31] also showed positive correlation of soil organic carbon with soil phosphorus. The results were in accordance to findings of Kaur et al. [25] that at late growing stages of crop fungal population showed negative correlation with soil potassium.On the reproductive stage, paddy was just at the maturity stage, the paddy roots grew slowly or even stagnated, which weakened the competition with soil microorganism and resulted in the increase of microbial biomass.

The amount of organic matter in soil imparts direct influence on biological activities of soil microflora [32]. Presence and mineralisation of organic matter influenced the soil physical, chemical and biological properties. Soil organic carbon was positively influenced by total bacterial and diazotrophic population, owing to the maximum positive correlation coefficient was observed between organic carbon and these populations. Whereas, fungal population showed positive impact (r=0.810, p=0.01) on organic carbon at tillering stage. Actinobacteria showed a significant positive impact on soil organic carbon (r=0.614, p=0.05) at reproductive stage. Interaction of organic carbon with fungal population remained positively correlated only up to tillering stage, whereas, it was found non-significant negative at reproduction and maturation stage. On contrary, Actinobacteria population showed reverse relationship with soil organic carbon. As indicated by the significant negative correlation upto tillering stage, but, non-significant positive correlation at later growth stage.

The results were in accordance with studies of Laldinthar and Dkhar [33] who studied the correlation between bacterial population and soil physico-chemical properties. They showed that bacterial population in the rhizospheric soil was positively correlated with organic matter content. Similar results were observed by Kaur et al. [25] that bacterial population had significant positive correlation with soil organic carbon content of soil. Similar results were reported by Setiawati [34] who studied the relationship between soil microbes and soil chemical properties and observed that bacterial population was positively correlated with organic carbon.

Table 1. Composition of different culture media used in study

S.no.	Medium	Composition	References
1	Nutrient agar	Beef extract (03.00g/l), Peptone (05.00 g/l), Sodium chloride (05.00 g/l), Agar (20.00 g/l), Distilled water (1000 ml)	Wright [8]
2	Glucose yeast extract medium	Glucose (10.00 g/l), Tri-calcium phosphate (05.00 g/l), Ammonium sulphate (00.50 g/l), Sodium chloride (00.20 g/l), Magnesium sulphate (00.10 g/l), Potassium chloride (00.20 g/l), Yeast extract (05.00 g/l), Manganese sulphate (0.001 g/l), Ferrous sulphate (0.001 g/l), Agar (20.00 g/l), Distilled water (1000 ml)	Mossel et al. [9]
3	Jensen medium	Sucrose (20.00 g/l), Dipotassium hydrogen ortho-phosphate (01.00 g/l), Magnesium sulphate heptahydrate (00.50 g/l), Sodium chloride (00.50 g/l), Sodium molybdate dihydrate (0.001 g/l), Calcium chloride dihydrate (00.01 g/l), Calcium carbonate (02.00 g/l), Agar (20.00 g/l), Distilled water (1000 ml)	Jensen [10]
4.	Kenknight's Medium	Dextrose (1.0 g/l), Monopotassium dihydrogen phosphate (0.10 g/l), Sodium nitrate (0.10 g/l), Potassium chloride (0.10 g/l), Magnesium sulphate (0.10 g/l), Agar (20.0 g/l), Distilled water (1000 ml)	Kenknight and Muncie [11]

Table 2. Correlation analysis of microbial population and soil enzymatic activities during different paddy growth stages

Paddy growth stages		BAC	FUN	ACT	DIAZO	DEHYD	UREASE	ALK P
At transplantation	**BAC**	1	0.487	-0.430	0.818**	0.885**	0764**	0.857**
	FUN		1	-0.450	0.016	0.469	-0.151	- 0.373
	ACT			1	-0.248	-0.720**	-0.090	-0.711**
	DIAZO				1	0.776**	0.963**	0.628*
	DEHYD					1	0.649**	0.876**
	UREASE						1	0.593*
	ALK P							1
Tillering stage	**BAC**	1	0.797**	-0.867**	0.748**	0.872**	0.866**	0.929**
	FUN		1	-0.773**	0.873**	0.942**	0.812**	0.639*
	ACT			1	-0.650**	-0.759**	-0.705**	-0.859**
	DIAZO				1	0.960**	0.957**	0.718**
	DEHYD					1	0.952**	0.785**
	UREASE						1	0.861**
	ALK P							1

Paddy growth stages		BAC	FUN	ACT	DIAZO	DEHYD	UREASE	ALK P
Reproductive stage	**BAC**	1	0.140	0.849**	0.822**	0.924**	0.403	0.845**
	FUN		1	0.059	0.304	0.389	0.437	-0.243
	ACT			1	0.652**	0.719**	0.159	0.560*
	DIAZO				1	0.948**	0.834**	0.734**
	DEHYD					1	0.695**	0.758**
	UREASE						1	0.430
	ALK P							1
Maturation stage	**BAC**	1	0.280	0.124	0.723**	0.849**	0.355	0.846**
	FUN		1	-0.184	0.193	0.621*	0.436	-0.139
	ACT			1	-0.423	-0.185	-0.633*	-0.127
	DIAZO				1	0.830**	0.844**	0.758**
	DEHYD					1	0.731**	0.603*
	UREASE						1	0.309
	ALK P							1

**Correlation is significant at 0.05 level (two tailed), **Correlation is significant at 0.01 level (two tailed)*

Table 3. Correlation analysis of microbial population and soil nutrient status during different paddy growth stages

	At transplantation				Tillering stage				Reproductive stage				Maturation stage			
	N	**P**	**K**	**OC**	**N**	**P**	**K**	**OC**	**N**	**P**	**K**	**OC**	**N**	**P**	**K**	**OC**
BAC	0.809**	0.946**	0.923**	0.915**	0.886**	0.945**	0.982**	0.928**	0.924**	0.846**	0.977**	0.898**	0.937**	0.905**	0.994**	0.928**
FUN	0.081	0.327	0.405	0.329	0.922**	0.783**	0.799**	0.810**	0.348	-0.262	-0.029	-0.142	0.462	-0.062	0.187	-0.046
ACT	-0.374	-0.597*	-0.548*	-0.670**	-0.863**	-0.959**	-0.920**	-0.949**	0.712**	0.748**	0.831**	0.614*	-0.027	0.013	0.164	0.028
DIAZO	0.986**	0.849**	0.664**	0.833**	0.942**	0.760**	0.673**	0.785**	0.962**	0.800**	0.836**	0.773**	0.839**	0.726**	0.694**	0.797**

**Correlation is significant at 0.05 level (two tailed), **Correlation is significant at 0.01 level (two tailed)*

4. CONCLUSION

The biological activities in soil such as growth and enzymatic activity associated with indigenous soil microorganism as well as chemical properties (available nutrients) of paddy rhizosphere were positively affected by different crop growth stages, application of green manure and increase in plant density. The study concluded that bacterial and diazotrophic population were the most active microbial group during the whole growing season of paddy, indicated by the significant positive correlation with soil enzymatic activities and soil available nutrients. However, fungal and actinobacterial population showed a variable and alternative trend with respect to each other during growth period. Fungal population showed significant positive correlation with enzyme activities and available nutrients at tillering stage. However, at reproductive stage actinomycetes population had a positive correlation with enzymatic activities and available soil nutrients. So, Indigenous microbial communities had effectively influenced by the plant growth, which consequently affects inter-relationship between the microbial populations as well as with their metabolic activities, resulting in a direct impact on soil chemical properties. Hence, this study rationalises the concept of plants acting as an intact part of complex bio-geochemical cycling, thus governing soil health as well as fertility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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