



Effect of green manure and plant density on correlation between rhizospheric bio-chemical properties and rice (*Oryza sativa*) yield

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ABSTRACT

The present investigation was carried out to evaluate the effect of application of green manure along with varying plant density on microbial population and soil enzymatic activities of rhizospheric soil as well as on yield of rice (*Oryza sativa* L.) during year 2013–14. Application of green manures @15 t/ha and increased plant density up to 44 plants/m² positively enhanced microbial population in the soil. Correlation among different microbial population, soil enzymatic activities and soil nutrient status in different treatments irrespective of the time intervals showed that the treatments with higher plant density along with green manure showed a significant positive correlation between dehydrogenase enzyme and bacterial as well as with fungal population. On contrary, treatment having sole application of inorganic fertilizer with 22 plants/m²; actinomycetes and bacterial population had significant positive correlation with dehydrogenase activity whereas fungal population showed a negative relationship. The soil urease and alkaline phosphatase activity had positive correlation with bacterial and fungal population in all treatments except the treatment with 22 plants/m² + recommended inorganic fertilizers. There was significant negative correlation between fungal population and diazotrophic population in all treatments. The plant height and various yield attributes were significantly higher in treatment having green manure (15 t/ha) + 44 plants/m² + recommended NPK. Thus, above finding revealed that the agronomic practices (green manure and increased plant density) significantly improved the interaction of microbial population and soil enzymatic activities in rhizospheric soil which in turn affect the soil fertility as well as rice yield.

Key words: Green manure, Microbial population, Plant density, Rice, Soil enzymatic activities

Rice (*Oryza sativa* L.) is very important and it represents the staple diet of the world's population, making it the most important food crop of the developing world. In present scenario adoption of the practices which maintains the soil health, keeps the production system sustainable and provides qualitative food are necessary for meeting the nutritional requirements of growing population. For productive and sustainable farming, it is essential to replenish nutrients drawn or lost from the soil. Green manuring is the most attractive and simplest alternative solution to this scenario, as, it can increase the cropping system sustainability by increasing soil bio-physical properties, organic matter, fertility, and nutrient retention as well as reducing soil erosion and global warming (Tajeda *et al.* 2008).

Land use activities; particularly related to agricultural practices, can have considerable impact on the size and activity of soil microbial community and biological health of soil (Das and Dkhar 2011). The inseparable plant

microorganism system undergoes short/long term fluctuation depending on agricultural practice, plant development stage and agro-ecological condition. Plants not only have an influence on living microorganisms (via root exudates) but also influence them after their death (plant debris). In both cases, great changes take place in the composition and activities of soil microflora. Microbes are essential in agriculture due to their role in many biogeochemical transformations in soil, such as in cycling mineral compounds, decomposing organic materials, promoting/suppressing plant growth and in various biophysical processes. The soil microbial biomass is fundamental for maintaining soil function because it represents the main source of soil enzymes that regulate transformation processes of elements in soil (Bohme and Bohme 2006). These biochemical properties provide rapid and accurate information on soil quality (Gil-Stores *et al.* 2005) as influenced by agronomic practice. Keeping the above view in consideration, the present investigation was undertaken to study the effect of green manure and plant density on inter relationship of microbial population, enzymatic activities and nutrient levels in soil as well as yield of rice crop.

MATERIALS AND METHODS

It was a part of long term field experiment (since 2001)

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at Agronomy Department research farm (30° 54.282' N and 75° 46.851' E at an elevation of 262 m above mean Arabian sea level) of the Punjab Agricultural University, Punjab, India. The mean maximum and minimum temperature during this study ranged between 30.6–44.1°C and 21–29.1°C, respectively. Mean rainfall was recorded 476.0mm with relative humidity in range of 72–83%.

The experiment was laid out in a randomized block design in triplicate. Sunn hemp (*Crotalaria juncea*) was used as green manure crop, nitrogen (urea) was applied in three split doses (at puddling; at 3rd and 6th weeks after transplantation) whereas the full dose of (NH₄)₂HPO₄ and muriate of potash were applied at transplanting. Total 24 irrigations were applied during crop growth period. Total 5 treatments were tested, viz. T1: 22 plants/m² + 180 N/ha (Farmer's practice); T2: 33 plants/m² + Recommended dose of NPK (120-30-30) kg/ha; T3: 33 plants/m² + Recommended NPK + Green manure (15 t/ha); T4: 44 plants/m² + Recommended NPK + Green manure (15t/ha); T5: 33 plants/m² + Fertilizer on soil test basis. The spacing between row and between plants was 15×15 cm; 15×20 cm; and 15 ×25 cm, corresponds to plant densities 44 plants/m², 33 plants/m² and 22 plants/m², respectively. Treatment T1 (farmer's practice) was control treatment.

Soil samples (0–15cm) were collected at different time intervals (0, 45, 90 and 120 days after transplantation) during 2013–14 *kharif* cropping season. Soil samples were analyzed for population of various soil microbial communities, viz. total bacteria, actinomycetes, diazotrophs, methanotrophs and fungi on their respective growth medium by serial dilution spread plate technique. The colonies with desired traits on different media were counted and recorded in colony forming units per gram dry soil. Soil enzymatic activity such as dehydrogenase, urease and alkaline phosphatase were analyzed using methods of Klein *et al.* (1971), Bremner and Douglas (1971) and Tabatabai and Bremner (1969), respectively.

Soil samples were air-dried under shade, pounded, sieved (<2 mm) and analyzed for available N, available P and available K as per the methodology of Subbiah and Asija (1956), Olsen *et al.* (1954) and Mervin and Peech (1950), respectively.

Various yield attributes and yield of rice were recorded at crop harvest. The mean values recorded for different observation and results were subjected to correlation analysis to analyze the inter relationship between the parameters irrespective of time intervals using SPSS 16.0v software. Data for yield attributes and crop yield was subjected to two factor analysis of variance (ANOVA) and difference between treatments were analyzed by Duncun's test using SPSS 16.0 software.

RESULTS AND DISCUSSION

Interrelationship of microbial population and enzymatic activities: Application of green manure and inorganic fertilization with varying plant density significantly affected the microbial flora and enzymatic activities in soil. In

treatment T1, the bacterial and diazotrophic population showed significant positive correlation with all enzymatic activities; dehydrogenase ($r=0.983$, $r=0.993$ @ $P=0.01$), urease ($r=0.751$, $r=0.825$ @ $P=0.01$) and alkaline phosphatase ($r=0.806$, $r=0.840$ @ $P=0.01$), respectively. In contrary, methanotrophic and actinomycetes population showed significant positive correlation only with dehydrogenase ($r=0.775$, $r=0.723$ @ $P=0.01$) and alkaline phosphatase activity ($r=0.927$, $r=0.672$ @ $P=0.01$), respectively (Table 1a). Whereas, fungal population had negative correlation with dehydrogenase ($r= -0.033$) and urease activity ($r= -0.593$ @ $P=0.01$). This might be due to the application of inorganic nitrogen in the soil which could have been decreased the C:N ratio of soil and thus fungal population in soil was found to be decreased.

In treatment T2, increase in plant density might increase the root exudates in the soil and thus can affect the microbial population and their enzymatic activities in soil. Fungal population showed a significant positive correlation with dehydrogenase ($r=0.660$ @ $P=0.05$,) and alkaline phosphatase activity ($r=0.909$, @ $P=0.01$) respectively (Table 1b). It was observed that methanotrophic and actinomycetes population showed a non-significant positive correlation with dehydrogenase and urease activity, respectively. In treatment T4, the methanotrophic population showed a non-significant negative correlation with urease activity. This indicated negative impact of increased urease activity on methanotrophic population in soil with application of inorganic nitrogen and green manure (Table 1c). Whereas, total bacteria had significant positive correlation with urease activity ($r=0.913$, @ $P=0.01$) in treatment T4, suggesting the role of bacteria in converting urea into ammonia by their urease activity. Actinomycetes population showed a negative correlation with dehydrogenase and urease activity indicating competition between other microbes and actinomycetes. The application of green manure increased the nutrient availability that might have stimulated the growth of fast growing microbial population and diminished the growth of relatively slow growing microbes such as actinomycetes. Kennedy and Stubbs (2006) reported that soil microbial communities often change more rapidly with management and environment. A significant positive correlation of urease activity was established with fungal and bacterial population, whereas, phosphatase activity showed a positive correlation with fungal population.

Interrelationship of microbial population and soil nutrient status: In treatment T1, total bacteria ($r= 0.758$ @ $P=0.01$) and methanotrophic population (0.797 @ $P=0.01$) had significant positive correlation with available nitrogen in the soil whereas it had non-significant positive correlation with fungal population (Table 1). Thus, results indicated that application of inorganic nitrogen might have lowered the C:N ratio of soil which ultimately favored the growth of bacteria. Available nitrogen showed negative correlation ($r=-0.256$) with actinomycetes population in treatment T2. Whereas, it was found that diazotrophic population had positive correlation with soil available nutrients (N, P and

Table 1 Correlation analysis of microbial population, enzymatic activities and available nutrient of soil in different treatments

	Bacteria	Fungi	Actino- mycetes	Methano- trophs	Diazo	Dehydro- genase	Urease	Alkaline phosphatase	Available N	Available P	Available K
<i>Treatment T1: 22 plants/m² + 180 N/ha (Farmer's practice)</i>											
Bacteria	1	.005	.791**	.780**	.973**	.983**	.751**	.806**	.758**	.833**	.290
Fungi		1	.507	.594*	-.055	-.033	-.593*	.362	.137	-.469	-.885**
Actinomycetes			1	.858**	.689*	.723**	.221	.672*	.470	.342	-.104
Methanotrophs				1	.763**	.775**	.288	.927**	.797**	.426	-.360
Diazotrophs					1	.993	.825	.840	.844	.895	.292
Dehydrogenase						1	.807**	.848**	.833**	.881**	.282
Urease							1	.506	.658*	.985**	.674*
Alkaline phosphatase								1	.947**	.624*	-.239
Available N									1	.741**	-.110
Available P										1	.579*
Available K											1
<i>Treatment T2: 33 plants/m² + Recommended dose of NPK (120-30-30) kg/ha</i>											
Bacteria	1	.704*	.181	.553	.984**	.978**	.899**	.786**	.862**	.979**	.458
Fungi		1	.751**	.953**	.695*	.660*	.368	.909**	.348	.585*	-.198
Actinomycetes			1	.905**	.140	.067	-.256	.460	-.309	.013	-.386
Methanotrophs				1	.527	.471	.151	.774**	.110	.407	-.247
Diazotrophs					1	.988**	.912**	.821**	.892**	.976**	.386
Dehydrogenase						1	.937**	.820**	.925**	.984**	.387
Urease							1	.591*	.990**	.959**	.598*
Alkaline phosphatase								1	.611*	.731**	-.177
Available N									1	.932**	.512
Available P										1	.521
Available K											1
<i>Treatment T4: 44 plants/m² + Recommended NPK + Green manure (15 t/ha)</i>											
Bacteria	1	.695*	-.115	.433	.990**	.772**	.844**	.957**	.802**	.961**	.354
Fungi		1	-.181	.531	.745**	.967**	.583*	.648*	.800**	.760**	-.314
Actinomycetes			1	.726**	-.043	-.064	-.622*	.079	-.618*	-.364	-.422
Methanotrophs				1	.523	.639*	-.080	.557	.058	.260	-.527
Diazotrophs					1	.821**	.792**	.961**	.782**	.943**	.248
Dehydrogenase						1	.592*	.737**	.781**	.796**	-.271
Urease							1	.700*	.937**	.939**	.569
Alkaline phosphatase								1	.661*	.871**	.262
Available N									1	.932**	.255
Available P										1	.358
Available K											1

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

K) in all treatments. The microbial biomass and population increased in random with increasing soil nutrient pools providing evidence that resource availability shaped the microbial communities (De Vries *et al.* 2012). Fungal population showed a significant positive correlation with available nitrogen ($r=0.800$ @ $P=0.01$) and phosphorus ($r=0.760$ @ $P=0.01$) in treatment T4. This indicated that application of green manure increased the carbon content of the soil and thus balancing the C:N ratio of soil, ultimately favoring growth of fungal population. Actinomycetes showed negative correlation with available soil nutrients. Population of methanotrophs was negatively correlated ($r=-0.683$, $r=-0.527$) with available potassium.

The study was in accordance to Wang *et al.* (2010) who stated that presence of nitrogen alleviates the activities and metabolism of microorganisms especially excretion of soil enzymes. Zhang *et al.* (2013) also suggested that microbial biomass and composition might be dependent on the nutrient sources, thus had positive correlation with available nutrients. It was suggested that nutrient additions can significantly affect the population, composition and function of soil microorganisms. Ahmad (2010) revealed that green manure legume contributed substantial amount of K and some P and micronutrients in addition to N.

Effect on fungal-bacterial ratio: Fungi and bacteria differ in their responses with changes in agricultural management practices. In present study, the incorporation of sunn hemp; which is low C:N ratio (18.9:1) manure, favors the growth of the bacterial population. The application of green manure in treatment T3 and T4 resulted in significantly lower fungal/bacterial ratio at all-time intervals as compared to other treatments. Lowest fungal/bacterial ratio of 0.06 was observed in treatment T3 and T4 at 0 DAT, whereas; the maximum was found in treatment T1 (Table 2). Throughout the growth season of rice, the maximum fungal/bacteria ratio was found at the time of crop harvest. This may be due to the presence of high C:N ratio cellulosic plant remains in the soil, which were attacked by soil fungi (high C:N ratio as compared to bacteria). So, fungal/bacterial ratio in fields incorporated with green manure remained dominated with bacterial population as compared to that devoid of green manure. Incorporation of plant residues into soil favored the growth of bacterial population owing to the increased

contact between the substrate and bacteria (Strickland and Rousk 2010). Also, the fact that bacteria having a smaller C:N ratio than fungi, usually demands foods rich in nitrogen as green manure and legume residues. Application of fertilizer (organic or inorganic) in soil favors the bacterial community, whereas a substrate with a relatively wide C:N ratio enhances growth of the fungal population (Malik *et al.* 2016).

Yield attributes and yield: The application of green manure and altered plant density had significantly improved the plant height, yield attributes and yield of the crop. The maximum plant height was recorded in treatment T4 which was significantly higher than that of the control (Table 2). The maximum number of effective tillers/m² (386) was recorded in treatment T4 which was significantly higher over other treatments. Number of branches/panicle (18) was observed to be higher in treatments T3 and T4, which were significantly higher over the treatment devoid of green manure application. In control, a significant decrease in number of branches/panicle (13) was recorded as compared to other treatments. Number of branches/panicle (15) in treatment T2 was at par with treatment T5. Owing to the non-significant difference between the treatment T3 and T4 for number of branches/panicle, it can be concluded that there was greater significance of green manure on plant growth factors than plant density. Maximum number of filled grains/panicle (136) was observed in plots having treatment T4 that was at par with treatment T3 (134). Maximum grain weight was recorded in treatment T4 which was significantly higher than other treatments. This might be due to application of green manure which increased the nutrient availability in soil and thus increased the growth of the plant. These findings were in accordance to results reported by Siavoshi *et al.* (2011), that organic fertilizer as well as chemical fertilizer in combination with organic fertilizer can increase yield attributes as compared to untreated plants.

Grain (7.325 t/ha) and straw yield (8.665 t/ha) was observed maximum in treatment T4 which was significantly higher over the grain (7.075 t/ha) and straw yield (8.390 t/ha) in treatment T3 (Table 2). Application of green manure had resulted in increased microbial population and its activities in soil (Kaur 2014), which positively influenced the physical, chemical and biological properties of soil and thus increased

Table 2 Effect of green manure and plant density on rhizospheric fungal to bacterial ratio and rice yield attributes

Treatment	Fungal to bacterial ratio				Yield attributes and yield								
	0 DAT	45 DAT	90 DAT	120 DAT	Plant height (cm)	Effective tillers/m ²	No. of branches/panicle	No. of filled grains/panicle	No. of unfilled grains/panicle	Thousand grain weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	
T1	0.19 ^a	0.15 ^a	0.29 ^a	0.47 ^a	98.8 ^c	288 ^c	13 ^d	108 ^c	30 ^e	21.1 ^e	6.190 ^e	7.345 ^c	
T2	0.13 ^c	0.15 ^c	0.20 ^c	0.22 ^c	101.5 ^c	338 ^c	15 ^c	128 ^c	24 ^{bc}	21.6 ^c	6.645 ^c	7.865 ^c	
T3	0.06 ^d	0.17 ^d	0.16 ^d	0.18 ^d	103.2 ^b	357 ^b	18 ^b	134 ^b	20 ^d	22.0 ^b	7.075 ^b	8.390 ^b	
T4	0.06 ^e	0.11 ^e	0.09 ^e	0.08 ^e	103.8 ^a	386 ^a	18 ^a	136 ^a	22 ^c	22.4 ^a	7.325 ^a	8.665 ^a	
T5	0.07 ^b	0.10 ^b	0.24 ^b	0.33 ^b	101.0 ^d	330 ^d	15 ^c	124 ^d	24 ^b	21.4 ^d	6.605 ^d	7.825 ^d	

DAT, Days after transplanting

the yield of crop. The results were in accordance to Selvi and Kalpana (2009) that continuous use of green manure before rice planting significantly increased the paddy yield as compared to non-green manured plots. The yields of rice was significantly ($P < 0.05$) greater in legumes than in the fallow-based rice-wheat plot (Zahir *et al.* 2011).

The application of green manure with increasing plant density and inorganic fertilizers had boosted the healthy interactions between the microbial communities in soil which imparted direct positive effect on the availability of nutrient in the soil and hence improved soil fertility as well as yield, along with satisfying all the criteria of sustainability.

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