Research Article

Effect of Green Manure and Bio-Inoculants on Inter-Relationship of Soil Biological Properties of *Kharif* Maize

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Abstract

Land use practices significantly influence the biological properties of soil rhizosphere as well their interaction with each other. In present study, different combination of inorganic, organic fertilizers and bioinoculants was assessed for their influence on correlation of soil microbial communities (total bacteria, fungi, actinomycetes, diazotrophs and P-solubilizers) and enzymatic activities (dehydrogenase, urease and alkaline phosphatase) in maize rhizosphere. The results showed a significant positive correlation of soil total bacterial population with dehydrogenase activity in all treatments; however the highest positive correlation was recorded for the treatment having combined application green manure, inorganic fertilizer and bioinoculant. Total fungal population was observed in negative correlation with bacterial population whereas it showed significant positive correlation with diazotrophic and P-solubilizing bacterial population. All soil enzymatic activities were in significant positive correlation with each other. Green manure application resulted into a negative correlation of all microbial populations with alkaline phosphatase and urease activity indicating that green manure had significantly increased the available nitrogen and phosphorous content in maize soil. On the other hand, dehydrogenase enzyme activity was observed in positive correlation with all microbial communities in the rhizospheric soil of maize. It was concluded that synergistic application of green manure and bio-inoculant altered the dynamics between soil microbial communities and enzyme activity in such a way that it resulted into improved soil health. Therefore, the application of potential bioinoculants with organic practices can be adopted as standard practice for sustainable maintenance of soil fertility.

Keywords: Correlation, Enzyme activities, Green manure, *Kharif* maize, Land use practices, Microbial communities

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Introduction

Cereals are the world's major source of food for human nutrition. In developing countries like India, it is very challenging to feed the growing population in a sustainable manner. But the degrading soil quality with excessive use of fertilizers necessitates modifying land use management practices that can improve soil quality and ensure sustainable agricultural productions. Replenishing the soil nutrient resources that has been removed or lost due to continuous cultivations can be the only approach to maintain the productivity and sustainability of agricultural system. The appropriate utilization of manures within management systems can increase levels of plant nutrients and enhance soil microbial biomass, activity and diversity [1, 2]. Hence, integrated farming can be the simplest alternative approach to cope with this scenario.

Any change in land use management practices, specifically in agricultural system can directly influence the interactions between microbial community structure and their function in soil. Microbes in plant rhizosphere; can undergo continuous fluctuations depending upon land management, ecological conditions and developmental stage of plant. Agricultural management practices, particularly the application of fertilizers; are known to have great impact on the size and structure of the soil microbial community. The composition and activities of microbial communities determine soil quality and fertility largely by regulating the biogeochemical cycles and turnover processes of organic matter. Soil quality has chemical, physical and biological aspect; and the latter being most responsive to rapid changes in soil conditions as compared to others [3]. Alteration in cultivation technique, soil properties and agronomic amendment can be directly assessed through soil enzymatic activities as they act as sensitive indicators of soil quality [4]. Increases in the fungi/bacteria ration have been linked to increases in soil C and ecological buffering capacity [5, 6] and in response to organic management [6] as well as various organic amendments, such as livestock manure [7], crop residue [8] and green manure [9]. So, it is important to know how microbial structure interacts with soil enzymatic activities in response to agricultural management practices.

Materials and Methods

A field experiment was conducted consequently for two years (*kharif* 2016 and 2017) at School of Organic Farming of the Punjab Agricultural University, Punjab, India. This was situated at an elevation of 292 m above mean sea level and lie at N 30°54.570' latitude and E 75°46.732' longitude under the Trans-Gangetic Plains' agro-climatic zone. The meteorological data during experimental period showed that weekly mean maximum air temperature ranged between 32.2 to 41.1 and 32.0 to 38.9 in year 2016 and 2017 respectively, while the weekly mean minimum temperature ranged between 24.7 to 29.2 and 21.0 to 30.6 during year 2016 and 2017 respectively. A total of 496.7 mm and 396.3 mm rainfall received in *kharif* season of 2016 and 2017, respectively. The maximum value of mean weekly rainfall was 152.4 mm (in 2016) and 100 mm (in 2017) received during 26^{th} and 35^{th} SMW, respectively. The mean weekly relative humidity ranged between 42-79.5 per cent and 42-79 during 2016 and 2017, respectively. The experiment was laid out in a random block design with three replications of each treatment. A total of 4 different combinations of inorganic fertilizer, organic amendment (with and without green manure) and bio-inoculant (with and without bio-inoculant) were made which are listed in **Table 1**. Sunn hemp (*Crotolaria juncea*) was used as green manure crop in the study. Nitrogen (110 kg/acre urea) was applied in three split doses whereas the full dose of phosphorus (di ammonium phosphate 55kg/acre) and potassium (muriate of potash 20 kg/acre) were applied at sowing of maize crop (var. PMH1). In situ incorporation of *Crotolaria juncea* (Sunn hemp) was done eight days before the sowing of maize crop (in green manured plots). The bioinoculant used in the experiment was bacterial consortium which has dual ability of degrading cellulose and plant growth promoting activities. The bio-inoculant was sprayed over 45 day old *Crotolaria* plants just before ploughing to boost its early degradation. Maize crop (variety – PMH1) was raised by following the crop management practices recommended in Package of Practices, PAU, Ludhiana.

Table 1 Different combinations of treatments used in the experiment

Treatments	
T1	100% rec. N
T2	Bio-inoculant + 100% N
T3	Green manure + 100% N
T4	Bio-inoculant + Green manure +100% N

Soil sampling and analysis

Soil samples were collected from different treatments during 2016 and 2017 *kharif* cropping season. Soil subjected to different managements was sampled at four different time intervals (0, 30, 60 days after sowing and at harvest) over the maize growth period and was analysed for different characters using standard protocols. Plants were uprooted from five random locations from each treatment. Loose soil was shaken off the roots and the soil that adhered strongly to the roots was carefully brushed from them and kept as rhizospheric soil. The five rhizospheric samples from each treatment were combined to form one representative sample and analysed.

Soil samples were analysed for population of various soil microbial communities such as total bacteria, fungi, actinomycetes, diazotrophs and PSB using serial dilution spread plate method. Soil samples were air-dried under shade, pounded to break up large clods, sieved (<2 mm), and analysed for soil enzymatic activities as dehydrogenase activity, alkaline phosphate activity, urease activity as per the methodology of Klein *et al* [10], Bremmer and Douglas [11] and Tabatabai and Bremner [12], respectively.

Statistical analysis

The mean values recorded for different parameters during both years. The data of two years was pooled and results were subjected to Pearson's correlation analysis that provide inter relationship between the biological parameters as affected with different treatments irrespective of time intervals using SPSS 16.0 software.

Result and Discussion

Correlation analysis between microbial communities and soil enzymatic activities

The present results revealed that any change in fertilizer regime significantly altered interaction within microbial communities and enzymatic activities in soil. In treatment T1, with only the inorganic fertilizers applied during maize cultivation, the bacterial population showed significant positive correlation with dehydrogenase (r=0.863 @ p=0.01) and alkaline phosphatase (r=0.741 @ p=0.05), however total bacterial population showed negative correlation with fungal, actinomycetes and diazotrophic population (Table 2a). Since, dehydrogenase activity is the measure of microbial respiration and a reliable index of microbial activity in soil [13], the positive correlation indicated that bacterial population was the predominant microbial group in the soil, while the negative correlation between microbial communities showed competition to flourish in the rhizospheric region. Fungal population showed significant positive correlation with diazotrophic (r=0.835 @ p=0.01) and P-solubilizing bacterial population (r=0.733 @p=0.05). Also, the diazotrophic population was found in significant positive correlation with PSB r=0.939 @p=0.01.

Table 2 Correlation analysis of microbial communities and enzymatic activities in rhizospheric soil of *kharif* maize in a) treatment T1, b) treatment T2, c) treatment T3 and d) treatment T4

a) Treatment T1								
	Bac	**Fun**	**Actino**	**Diazo**	**Psb**	**Dehyd**	**Ap**	**Urease**
Bac	1							
Fun	-0.608	1						
Actino	-0.024	0.443	1					
Diazo	-0.094	0.835**	0.381	1				
Psb	0.086	0.733*	0.634	0.939**	1			
Dehyd	0.863**	-0.123	0.274	0.410	0.577	1		
Ap	0.741*	-0.012	0.369	0.452	0.622	0.920**	1	
Urease	0.642	0.039	0.391	0.445	0.606	0.830**	0.980**	1
b) Treatment T2								
	Bac	**Fun**	**Actino**	**Diazo**	**Psb**	**Dehyd**	**Ap**	**Urease**
Bac	1							
Fun	-0.426	1						
Actino	0.052	0.413	1					
Diazo	0.140	0.813**	0.275	1				
Psb	0.025	0.887**	0.584	0.934**	1			
Dehyd	0.884**	0.046	0.285	0.571	0.489	1		
Ap	0.788*	0.080	0.350	0.531	0.488	0.913**	1	
Urease	0.680*	0.067	0.329	0.449	0.423	0.788*	0.966**	1
c) Treatment T3								
	Bac	**Fun**	**Actino**	**Diazo**	**Psb**	**Dehyd**	**Ap**	**Urease**
Bac	1							
Fun	-0.290							
Actino	0.240	0.569	1					
Diazo	0.141	0.867**	0.468					
Psb	0.422	0.739*	0.780*	0.882**	1			
Dehyd	0.986**	-0.133	0.311	0.304	0.555			
Ap	-0.257	-0.812**	-0.856**	-0.844**	-0.969**	-0.390	1	
Urease	0.599	-0.745*	-0.365	-0.438	-0.303	0.502	0.459	1
d) Treatment T4								
	Bac	**Fun**	**Actino**	**Diazo**	**Psb**	**Dehyd**	**Ap**	**Urease**
Bac	1							
Fun	-0.395	1						
Actino	0.250	0.511	1					
Diazo	0.120	0.854**	0.573	1				
Psb	0.307	0.747*	0.784*	0.946**	1			
Dehyd	0.996**	-0.423	0.169	0.101	0.266	1		
Ap	-0.367	-0.671*	-0.829**	-0.884**	-0.976**	-0.320	1	
Urease	0.531	-0.798**	-0.422	-0.536	-0.463	0.565	0.410	1

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
[bac: total bacteria; Fun: Total fungi; Actino: actinomycetes ; Diazo: Diazotrophs; PSB-Phosphorous solubilizing bacteria; Dehyd: Dehydrogenase activity; Ap: alkaline phosphatase activity]

In the treatment T2, inorganic fertilizer application was integrated with cellulose degrading plant growth promoting bioinoculant. Correlation analysis indicated that total bacteria had positive relationship with all other microbial communities except fungal population. This might be due to lesser fungal to bacterial population in the soil rhizosphere (data not presented). Bacterial population actively participated in active pool of soil and nutrient transformation as it had significant positive correlation with dehydrogenase (r=0.884 @ p=0.01), alkaline phosphatase and urease i.e., r=0.788; r=0.680 @p=0.05 respectively (Table 2b). As, the total bacteria population had positive correlation with urease activity in both treatment T1 (r=0.642) and T2 (r=0.680 @ p=0.05) respectively, it suggested the role of bacteria in converting urea into ammonia by their urease activity. Dehydrogenase activity was observed in significant positive correlation with both alkaline phosphatase and urease activity, which could be related to increased microbial population due to bioinoculation and thus; their activities for the release of nutrients either present already in soil or fixed from the fertilizer.

Application of green manure in soil significantly improved the available nutrient pool in soil as it is rich source nitrogen and phosphorous. In treatment T3, having green manure with inorganic fertilizer, the correlation of total bacterial population was found significantly higher (r=0.986 @ p=0.01) with soil dehydrogenase activity (Table 2c). Fungal population showed a positive correlation with diazotrophic and PSB population. However, fungal population showed significant negative correlation with dehydrogenase activity indicating that total bacteria were the metabolically active group in soil. This result can be justified in terms that application of low C/N ratio organic matter favours the growth of organism having low C/N ratio (bacteria 8:1) as compared to organisms having higher ratio (Fungi 25:1). Bacterial population showed positive correlation with alkaline phosphatase activity whereas negative correlation with urease activity. Diazotrophic and P-solubilizing bacterial population had significant negative correlation with urease (r= -0.844) and alkaline phosphatase (r=-0.969) activity at 0.01 level of significance. This negative correlation might be the result of fact that alkaline phosphatase and urease activity usually stimulates in soils with low nutrient availability; while application of green manure is known to improve available pool of nitrogen and phosphorous in soil. However, application of green manure with addition of bioinoculants (Treatment T4) further increased the positive correlation of different parameters (Table 2d). Results were supported by Chang et al [4] that total bacterial community were closely related to all soil enzyme activities except for urease activity. Application of green manure significantly affected the functionality of various microbial communities in soil. Baligar et al [14] reported that enzymatic activities have close relationship with soil organic carbon and thus might have promoting effects on microbial activities. The present results were also supported by Kennedy and Stubbs [15] that different microbial communities in soil often respond rapidly to any alternation in land management and environmental conditions that ultimately had effect on their functionality in soil as these diverse microbial communities are associated with nutrient transformation and decomposition. Zhang et al [16] also indicated that organic amendments significantly enhanced enzyme activities and altered microbial community at the same time. They suggested that along with positive effects of organic manures, differences in organic matter composition and thus substrate availability were the most probable reasons for the differences in microbial community structure. Jha *et al.* [17] observed that the bacterial population showed a significant correlation ($r = 0.639, P < 0.05$) with dehydrogenase enzyme in the forest ecosystem. A significant positive correlation of urease activity was established with fungal and bacterial population, whereas, phosphatase activity showed a positive correlation with fungal population.

Conclusion

Soil microbial processes play crucial role for plant nutrient supply, so have a central role in nutrient dynamics and organic matter decomposition in soil. Management of soil fertility through organic fertilizers has always been a pivotal principle of sustainable agriculture. Yet, the impacts of these fertilizers on soil microbial community structure as well as function can vary widely. The major nutrient drivers in soil under organic fertilizers can be better understood by measuring impact of fertilizer regimes on microbial processes. So, in present study application of green manure (low C/N ratio) organic matter along with inorganic fertilizer stimulated the bacterial growth and function as compare to fungal population in soil. The altered fertilizer regimes imposed a significant impact on inter-relationship of microbial communities and enzymatic activities in response to altered nutrient availability in soil due to application of nitrogen rich green manure.

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