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Research Article

Long Term Application of Rice Straw and Nitrogen Fertilizer Affects Soil Health and Microbial Communities

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Abstract

Quantitative and representative recovery of micro-organisms from samples is necessary in understanding ecosystem function. Present study focused on application of rice straw and nitrogen fertilizer affects on microbial population and enzymatic activities in wheat field. Different soil microorganisms were enumerated at different time intervals (0, 45, 90 & 120 DAS). Rice straw application significantly enhanced the microbial count with the increasing number of days. Maximum total bacterial population (202×10^{7} cfu/g of soil), diazotrophic count (118×10^{5} cfu/g of soil), fungal count (78×10^{4} cfu/g of soil) and actinomycetes count (138×10^{5} cfu/g of soil) were observed at 45 DAS. The highest total bacterial count and fungal count was observed with 7.5 t/ha rice straw + 120 kgN/ha while diazotrophs and actinomycetes population were observed with 10 t/ha rice straw. Dehydrogenase, urease and phosphatase enzymatic activities were found significantly increased in organic amended soil samples. At 45 DAS, dehydrogenase enzyme (2.396 µg TPF/g soil/hr), urease (301.24 µg/g soil/hr) and alkaline phosphatase (12.403 µg/g soil/hr) were found to be the maximum with 7.5 t/ha rice straw + 120 kg N/ha. Correlation analysis showed that microbial population was correlated with soil enzymes, but diazotrophs were negatively correlated with nitrogen fertilizer.

Keywords: Dehydrogenase, organic fertilization, phosphatase, soil microbial population and urease

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Introduction

Soil quality greatly influences the capacity of the soil to meet the increasing demand of the higher productivity. Due to higher rate of turnover and repeated cultivation soil become deficient in nutrients. The chemical fertilizers are widely used to improve the quality of soil. These agrochemicals even though increase yield, make the microbial and plant systems more vulnerable to various stresses beside have deleterious effect on the environment. Because of the effect of synthetic chemicals and the associated quality, some ecologist and environmentalists have promoted the other extreme i.e., to demand agricultural commodities produced in accordance with specialized system that is totally independent of any form of synthetic chemical influence [1]. Practices are emerging to utilize the agricultural waste as organic fertilizer. Recycling of organic wastes in agriculture is much needed and returns organic matter into the soils [2].

The implication of rice straw as organic fertilizer enhances the population of soil micro-organisms. Fertilizers can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil [3]. Microbial diversity and activity are sensitive indicators that reflect the sustainability and productivity of terrestrial agroecosystems [4-6]. Soil fertility intensively depends upon soil micro-organisms activity as they help in various biochemical transformations and also act as sink and source of nutrients. Soil microorganisms slowly break down the organic material into an inorganic and water soluble form which the plants can use [7].

Addition of organic amendments and adoption of management practices that increase soil organic matter also lead to increase soil enzymatic activities. Soil enzymes increase the reaction rate at which plant residues decompose and release plant available nutrients. Enzyme activity is the cumulative effect of long term microbial activity and activity of the viable population at sampling (USDA Natural Resources Conservation Service).

Effect of long-term application of organic fertilizers on soil microbial populations can be measured either as changes in the population of a particular organism, organism groups or methodologically defined pools such as the microbial biomass or as changes in biological activity, for example, soil respiration and enzyme activities. Variable effects of a given amendment on different organisms may change the composition of the microbial community without changing total populations or activities [8]. The purpose of this study was to assess the population dynamics of resident soil micro-organisms in relation to the period of organic fertilizer application.

Material and Methods

A long term experiment was conducted to study the effect of rice straw and N fertilizer on microbial communities and soil enzymatic activities. Field experiment was laid down since 2005 using standard agronomic practices for cultivation of wheat at Punjab Agricultural University, Ludhiana. The different doses of rice straw (0, 5, 7.5 and 10 t/ha) were incorporated in split plot design along with the addition of different N-levels (0, 90,120 and 150 kgN/ha). Soil samples were collected from 0-15 cm depth by using auger from wheat rhizosphere at 0, 45 DAS, 90 DAS and 120 DAS (days after sowing) in triplicates. The colony forming unit (cfu) per gram count of different soil microbes viz. bacteria, diazotrophs (N-fixers), fungi and actinomycetes were enumerated by standard serial dilution spread plating technique on different media such as Nutrient Agar [9], Burk's [10], Glucose Yeast Extract and Kenknight's respectively and soil samples were also analysed for different soil enzyme activities (such as dehydrogenase, urease and alkaline phosphatase). Dehydrogenase activity was determined by method of Mersi and Schinner [11], Urease activity was estimated by Mcgarity and Myers method of McGarity *et al* [12] and Alkaline Phosphatase activity was assayed by method of Bessey *et al* [13].

Treatments

T1	Without Rice Straw	+	Without N Fertilization
T2	5 ton/ha Rice Straw	+	Without N Fertilization
T3	7.5 ton/ha Rice Straw	+	Without N Fertilization
T4	10 ton/ha Rice Straw	+	Without N Fertilization
T5	Without Rice Straw	+	90 kg N/ha
T6	5 ton/ha Rice Straw	+	90 kg N/ha
T7	7.5 ton/ha Rice Straw	+	90 kg N/ha
T8	10 ton/ha Rice Straw	+	90 kg N/ha
T9	Without Rice Straw	+	120 kg N/ha
T10	5 ton/ha Rice Straw	+	120 kg N/ha
T11	7.5 ton/ha Rice Straw	+	120 kg N/ha
T12	10 ton/ha Rice Straw	+	120 kg N/ha
T13	Without Rice Straw	+	150 kg N/ha
T14	5 ton/ha Rice Straw	+	150 kg N/ha
T15	7.5 ton/ha Rice Straw	+	150 kg N/ha
T16	10 ton/ha Rice Straw	+	150 kg N/ha

Statistical Analysis

Microsoft Excel 97-2003 was used in the statistical processing of the data. Analysis of Variance (ANOVA) for soil microbial population and soil enzymatic activities was analyzed using Statistical version 3. Simple correlation was performed to test the relationships among variables.

Results and Discussion

Microbial populations

In present investigation different microbes were enumerated to study the impact of different doses of rice straw (RS) along with different N-levels in the rhizospheric soil of wheat crop. The soil samples were enumerated at different time intervals and were observed to be significantly higher than samples having no RS and no nitrogen (T1) i.e control (**Table 1**). At 45 DAS, the maximum total bacterial population was found with treatment having 7.5 t /ha RS and 120 kgN/ha i.e. T11 (202×10^7 cfu/g of soil) followed by T16 (184×10^7 cfu/g of soil) which in turn was followed by T15 (178×10^7 cfu/g of soil). Similarly, the highest fungal population at 45 DAS was exhibited by T11 (78×10^4 cfu/g of soil) followed by T16 (75×10^4 cfu/g of soil) and T15 (45×10^4 cfu/g of soil). However, the samples were enumerated for diazotrophic count and found maximum at 45 DAS with treatment having 10 t/ha RS and no nitrogen i.e. T4 (118×10^5 cfu/g of soil) followed by T3 (112×10^5 cfu/g of soil) which in turn was followed by T2 (109×10^5 cfu/g of soil). Similarly, the actinomycetes count at 45 DAS observed to be the maximum with treatment T4 (138×10^5 cfu/g of soil) followed by T3 (132×10^5 cfu/g of soil) and T2 (130×10^5 cfu/g of soil). The present investigation showed that the total bacterial count and fungal count with 10 t/ha RS and 7.5 t/ha RS were statistically at par but, statistically higher than 5 t/ha RS. It was also observed that bacterial and fungal population with 150 kg N/ha and 120 kg N/ha along with different doses of rice straw were statistically at par but, significantly higher than samples having 90 kgN/ha. However, diazotrophic count and actinomycetes count found to be lower with higher doses of N-level while the count was significantly higher with higher doses of RS (without nitrogen).

Table 1 Effect of incorporation of rice straw and inorganic fertilization on different soil microorganisms population at different time intervals

Treatments	Bacterial count (CFU/ g × 10^6)				Fungal count (CFU/ g × 10^3)				Diazotrophic count (CFU/ g × 10^4)				Actinomycetes count (CFU/ g × 10^5)			
	0 DAS	**45 DAS**	**90 DAS**	**120 DAS**	**0 DAS**	**45 DAS**	**90 DAS**	**120 DAS**	**0 DAS**	**45 DAS**	**90 DAS**	**120 DAS**	**0 DAS**	**45 DAS**	**90 DAS**	**120 DAS**
T1	28± 0.94	62± 0.76	48± 0.76	32± 1.21	0.5± 0.94	28± 1.00	10± 0.82	5± 0.82	35± 0.94	58± 1.33	46± 0.82	38± 0.82	22± 1.21	74± 0.82	48± 0.76	34± 0.94
T2	34± 0.58	95± 0.76	59± 1.21	38± 1.44	0.7± 0.76	42± 1.33	28± 1.07	8±0.58	58± 1.36	109± 1.07	75± 1.55	62± 1.33	60± 1.24	130± 1.21	92± 0.82	84± 0.76
T3	42± 1.21	99± 0.94	65± 0.76	49± 0.76	0.9± 0.94	52± 0.94	20± 1.10	10± 0.82	62± 0.94	112± 1.10	77± 1.24	65± 0.94	62± 0.82	132± 1.15	98± 0.82	90± 0.94
T4	46± 0.58	97± 0.58	68± 1.21	52± 0.82	1.0± 1.21	50± 0.76	22± 0.82	10± 0.94	66± 0.82	118± 1.33	85± 0.94	68± 0.94	64± 1.10	138± 1.70	106± 1.21	92± 1.24
T5	37± 0.94	87± 1.53	62± 1.33	40±0.76	1.1± 0.82	48± 0.76	25± 1.10	12± 0.94	34± 1.42	76± 0.82	55± 1.24	42± 0.82	30± 1.24	92± 1.10	61± 1.42	57± 0.82
T6	48± 2.24	103± 1.44	68± 1.42	47± 1.33	1.3± 1.33	56± 0.82	28± 0.76	15± 1.32	47± 1.24	102± 0.58	78± 0.94	64± 0.82	39± 0.76	105± 1.10	68± 1.07	63± 1.21
T7	50± 0.94	109± 1.07	71± 0.82	56± 1.33	1.7± 0.82	59± 0.82	29± 0.9	15± 1.07	51± 1.10	105± 0.94	79± 0.82	65± 0.82	50± 0.76	118± 1.10	80±0.94	73± 0.82
T8	66± 1.07	117± 1.10	78± 0.82	62± 0.82	1.8± 0.94	58± 1.33	32± 0.82	18± 0.82	54± 0.94	105± 1.10	83± 1.70	69± 0.82	58± 0.76	128± 0.94	90± 1.24	79± 0.82
T9	62± 0.76	112± 1.33	80± 0.76	64± 0.82	1.7± 0.82	60± 0.94	34± 1.96	18± 0.58	31± 1.33	81± 1.33	56± 1.07	44± 1.15	28± 1.10	90± 0.82	59± 0.94	44± 0.82
T10	79± 0.82	138± 1.33	106± 0.94	82± 0.94	2.0± 0.94	72± 0.94	45± 1.44	22± 1.10	45± 0.94	91± 0.58	62± 1.21	50± 0.94	34± 1.33	103± 1.33	65± 0.82	60± 0.58
T11	86± 1.61	202± 1.42	154± 0.82	137± 1.10	2.4± 0.94	78± 0.76	49± 1.00	28± 1.33	48± 0.94	95± 0.76	65± 0.82	56± 1.10	48± 0.82	112± 1.07	78± 1.10	68± 1.33
T12	78± 1.33	168± 0.82	134± 1.33	118± 0.82	2.2± 1.21	76± 1.10	44± 0.82	25± 1.33	48± 1.15	98± 0.82	66± 1.33	54± 1.07	56± 1.21	120± 1.52	88± 0.82	78± 1.07
T13	59± 0.94	114± 0.82	73± 1.10	58± 0.82	1.4± 1.21	62± 0.82	36± 0.94	20± 0.94	32± 1.33	78± 1.33	54± 1.53	40± 1.55	25± 0.94	86± 0.82	56± 1.33	42± 0.94
T14	70± 0.82	129± 1.24	101± 1.10	83± 1.21	1.8± 0.58	70± 0.82	42± 0.82	20± 1.10	32± 1.33	83± 1.33	57± 0.58	46± 1.00	32± 0.82	102± 1.07	64± 0.94	57± 1.07
T15	81± 0.58	178± 0.58	132± 0.82	112± 0.82	2.0± 0.82	72± 0.82	46± 0.82	24± 1.32	35± 1.33	88± 1.00	62± 0.58	47± 1.33	44± 1.21	114± 1.70	77± 1.33	64± 0.82
T16	84± 0.76	184± 0.82	142± 1.21	126± 1.33	2.2± 0.94	75± 1.21	48± 0.58	26± 0.82	38± 1.00	92±0.94	64± 0.82	48± 1.21	55± 0.82	122± 0.76	82± 0.58	76± 0.82
CD (5%)	Treatments: 3.422 Interaction: 5.023				Treatments: 2.630 Interaction: 4.577				Treatments: 1.522 Interaction: 3.922				Treatments: 2.293 Interaction: 4.641			

Soil microbial biomass has been used as an index of soil fertility which depends on nutrient fluxes. The organic fertilizers generally increased the microbial number but the increase depends upon the application rate or addition of other chemical fertilizer [14]. Krishnakumar *et al* [15] revealed that organic material significantly increased the bacterial population. Significant impact of straw incorporation along with inorganic N on bacterial population in the soil was evidenced at different growth stages by Vijayaprabhakar *et al* [16]. Amendment of synthetic nitrogen fertilizer could affect the activity of diazotrophs was reported by Sarkar *et al* [17] and he also reported that nitrogen fertilizer affects the plant association with the diazotrophic population in nitrogen amended samples. Tapia-Herandez *et al* [18] and Muthukumarsway *et al* [19] demonstrated that addition of compost (organic fertilizer) helped in the proliferation of diazotrophic bacteria while nitrogen fertilization inhibited the proliferation of diazotrophs. The decrease in actinomycetes population in treatment amended with nitrogen fertilizers were also reported by Nakadii [20]. The micro-organisms were inclined to decrease in vial cell number over the course of winter, maintain a high level at 45 DAS and drop thereafter (90 DAS and 120 DAS). The findings of Gaind and Nain [21] also concluded that drastic decrease in microbial biomass may be attributed to low availability of substrate at crop maturity stage.

Enzymatic activity

The different soil enzymes were analysed from the same rhizospheric soil samples taken at different time intervals. The enzymatic activities observed to be increased when compared to control 0 DAS enzymatic activities. The dehydrogenase enzyme which is an intracellular enzyme (**Figure 1**) found to be maximum at 45 DAS with T11 (23.96 µg TPF/g soil/hr) followed by T16 (21.65 µg TPF/g soil/hr) which in turn followed by T15 (19.95 µg TPF/g soil/hr). Similarly, urease activity at 45 DAS (**Figure 2**) was showed maximum with T11 (329.96 µg/g soil/hr) followed by T16 (326.42 µg/g soil/hr) and T15 (324.63 µg/g soil/hr). The alkaline phosphatase at 45 DAS (**Figure 3**) was analysed and maximum activity was observed with T11 (14.772 µg/g soil/hr) followed by T16 (14.679µg/g soil/hr) followed by T15 (14.651 µg/g soil/hr). The present study revealed that soil enzymatic activities with 10 t/ha RS and 7.5 t/ha RS were statistically at par but, statistically higher than 5 t/ha RS. It was also recorded that soil enzymatic activities with 150 kg N/ha and 120 kg N/ha along with different doses of rice straw were statistically at par but, significantly higher than samples having 90 kgN/ha with different doses of rice straw.

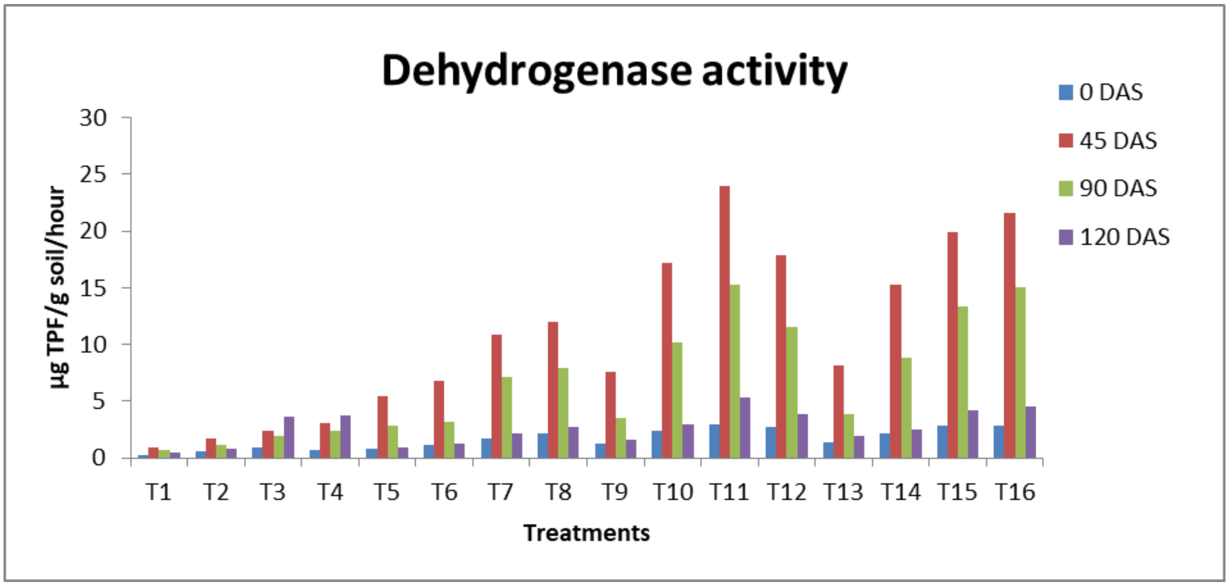

Figure 1 Effect of incorporation of rice straw and inorganic fertilization on soil dehydrogenase activity

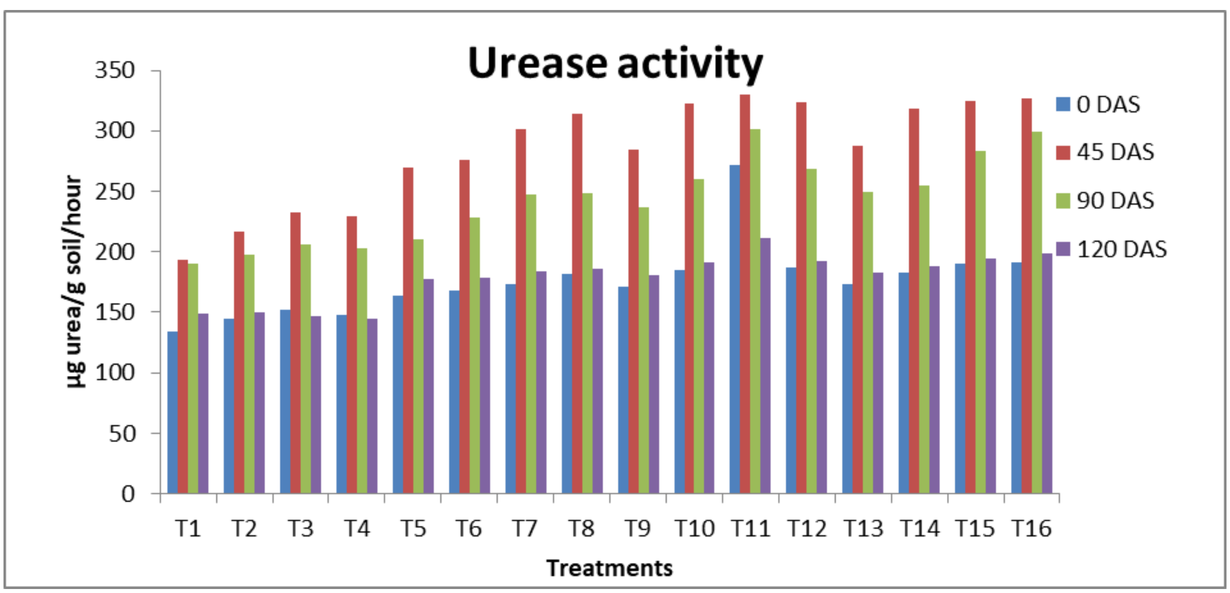

Figure 2 Effect of incorporation of rice straw and inorganic fertilization on soil urease activity

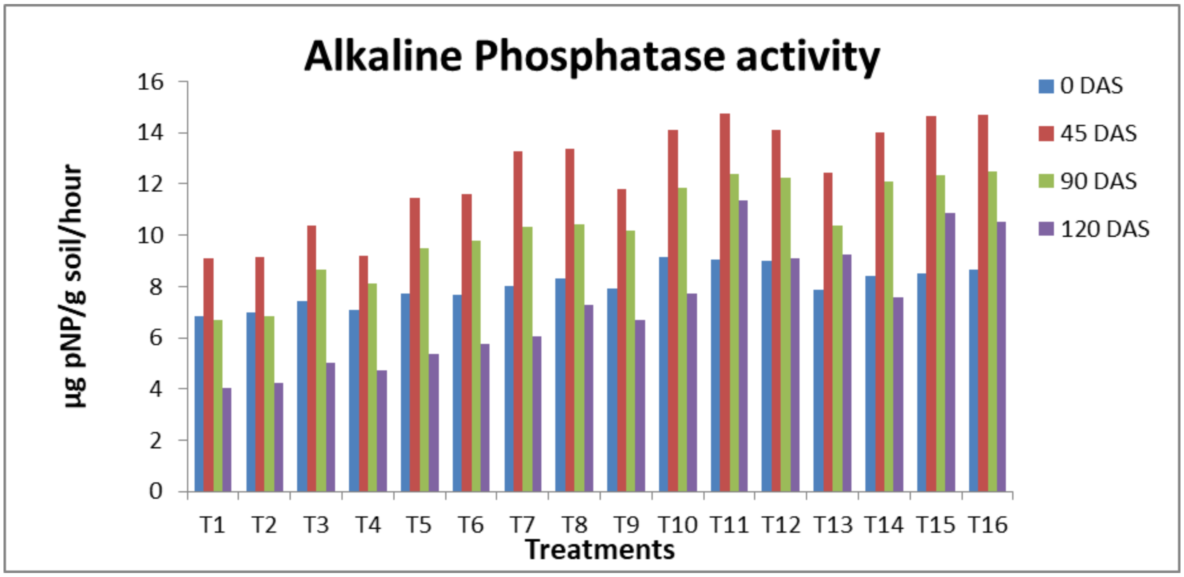

Figure 3 Effect of incorporation of rice straw and inorganic fertilization on soil alkaline phosphatase activity

Soil enzymes, which have been shown to be related to microbial activity, catalyse reactions in soils that are important in nutrient cycling [22]. Garg and Bahl [23] also reported that straw incorporation has significant roles in improving the activity levels of soil enzymes and microbial biomass communities. According Bandick and Dick [24] residue incorporation in the soil can increase the activity levels of various soil enzymes, and the levels are usually higher than those after treatment with chemical fertilizers alone [25]. The dehydrogenase activity, urease activity and phosphatase activity were found to be higher with rice straw treated (having N-levels) soil samples and a decline in soil enzymatic activities towards crop maturity was observed. Present results were similar with the findings of Gaind and Nain [21] who concluded that the decrease may be associated to low microbial count related to poor availability of substrate to sustain microbial biomass. Low enzymatic activity in soil during winter compared to warmer season was also reported by Ross et al [26].

Correlation analysis

The correlation analysis was studied among different soil microbes and different soil enzymatic activities at different time intervals (**Table 2, 3 & 4**). At 45 DAS, bacterial population was highly correlated to phosphatase (r=0.833), dehydrogenase (r=0.910) and urease (r=0.655) activities at 0.01 level of significance (**Table 3**) when compared to 0 DAS microbial count. Fungal population was positively correlated with, phosphatase (r=0.889) dehydrogenase (r=0.893) and urease (r=0.749) activities at 0.01 level of significance. Actinomycetes count was statistically non-significantly correlated with phosphatase (r=0.019), urease (r=0.026) and dehydrogenase (r=0.124) activities at 0.01 level of significance. Diazotrophic population was negatively correlated with alkaline phosphatase (r=-0.312), dehydrogenase (r=-0.248) and urease (r=-0.193) activities at 0.01 level of significance. At 90 DAS and 120 DAS, correlation between microbial population and enzyme activities showed that bacteria, and fungal populations were strongly correlated with all the three enzyme activities viz., alkaline phosphatase, urease and dehydrogenase at 0.01 level of significance (**Tables 4** and **5**). However, actinomycetes population showed non-significant correlation with enzyme activities while diazotrophs showed negative correlation with nitrogen fertilizer.

Table 2 Correlation analysis between microbial population and enzymatic activities at 0 DAS

Correlation of variables at 0 DAS							
	Bacteria	**Diazotrophs**	**Actinomycetes**	**Fungi**	**Phosphatase**	**Dehydrogenase**	**Urease**
Bacteria	1	-.456**	.162	.876**	.872**	.952**	.815**
Diazotrophs		1	.609**	-.329*	-.397**	-.415**	-.270
Actinomycetes			1	.122	.002	.154	.057
Fungi				1	.875**	.888**	.776**
Phosphatase					1	.946**	.794**
Dehydrogenase						1	.804**
Urease							1

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

Table 3 Correlation analysis between microbial population and enzymatic activities at 45DAS

Correlation of variables at 45 DAS							
	Bacteria	**Diazotrophs**	**Actinomycetes**	**Fungi**	**Phosphatase**	**Dehydrogenase**	**Urease**
Bacteria	1	-.195	.228	.882**	.833**	.910**	.655**
Diazotrophs		1	.797**	-.381**	-.312*	-.248	-.193
Actinomycetes			1	.000	.019	.124	.026
Fungi				1	.889**	.893**	.749**
Phosphatase					1	.895**	.823**
Dehydrogenase						1	.699**
Urease							1

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

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

Table 4 Correlation analysis between microbial population and enzymatic activities at 90 DAS

Correlation of variables at 90 DAS							
	Bacteria	**Diazotrophs**	**Actinomycetes**	**Fungi**	**Phosphatase**	**Dehydrogenase**	**Urease**
Bacteria	1	-.369**	.135	.861**	.846**	.915**	.860**
Diazotrophs		1	.686**	-.505**	.389**	-.310*	-.347*
Actinomycetes			1	-.110	-.062	.085	-.003
Fungi				1	.886**	.823**	.854**
Phosphatase					1	.845**	.880**
Dehydrogenase						1	.848**
Urease							1

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

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

Table 5 Correlation analysis between microbial population and enzymatic activities at 12 0 DAS

Correlation of variables at 120 DAS							
	Bacteria	**Diazotrophs**	**Actinomycetes**	**Fungi**	**Phosphatase**	**Dehydrogenase**	**Urease**
Bacteria	1	-.503**	.154	.856**	.901**	.576**	.656**
Diazotrophs		1	.574**	-.471**	-.639**	.017	-.363*
Actinomycetes			1	.026	-.069	.365*	-.123
Fungi				1	.876**	.673**	.706**
Phosphatase					1	.480**	.685**
Dehydrogenase						1	.363*
Urease							1

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

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

Conclusion

The present study was attempted to assess the effect of organic and nitrogen fertilizers on microbial communities and soil enzymatic activities in wheat field. It was observed that long-term application of rice straw along with N-fertilizers significantly improves the microbial population and enzymatic activity of the soil in the wheat field. The rice straw dose of 7.5 t/ha and nitrogen @ 120 kg/ha was found to be best application for improving soil microbial population and hence improved soil enzymatic activities.

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