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Correlation of Methanotrophs and Soil Enzymes with Available Nutrients in Long Term Green Manured Rice Rhizospheric Soil

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Authors' contributions

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

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ABSTRACT

The indiscriminate use of chemical fertilizers in agriculture decreases the soil fertility as well as soil health and their adverse effects are clearly visible on soil microflora, soil function and structure. This scenario necessitates the need to adopt the integrated organic farming which maintains the soil health and sustainability. So, the present study was conducted during *kharif* season 2013 at Department of Microbiology and Agronomy, Punjab Agricultural University, Punjab, India to assess the effect of green manure application along with variations in plant density on methanotrophic populations, soil enzymatic activity and their correlation with available nutrient status of the soil. Highest methanotrophic population (176×10^5 cfu/g) was observed in the treatment having green manure (15 t/ha) + 44 plants/m^2 + recommended NPK at 90 DAT. The highest soil dehydrogenase (50 µg Triphenyl formazon/g soil/hr) and urease activity (855 µg/g soil/h) was found in the treatment having green manure (15 t/ha) + 33 plants/m^2 + recommended NPK whereas, alkaline phosphatase (21.9 µg p-nitro phenol/g soil/h) were observed in the treatment having green manure+ 44 plants/m^2

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+ recommended NPK. A highly significant positive correlation was observed between methanotrophs and available nutrient content in soil. Similarly, the soil enzymatic activities were also positively correlated with the soil nutrients and organic carbon in soil. The study suggested that application of green manure along with increased plant density improves the soil health and fertility hence, increases the sustainability of cropping system.

Keywords: Green manure; methanotrophic population; plant density; rice; soil enzymatic activities.

1. INTRODUCTION

The first two decades of the green revolution increased the productivity of crops on account of increasing nutrient-use efficiency. Afterwards, crop productivity began to decline due to imbalances in the use of chemical fertilizers along with deficiencies of micro-nutrients in the soil [1]. Nowadays, a major agricultural research priority is to develop sustainable agriculture which can increase the soil's productivity and plant growth by the correct utilization of nutrient sources. The sustainability of cropping systems involves enhancing soil ecosystem processes and biological interaction along with judicial use of agrochemical and fertilizer [2]. Organic fertilizers have always been a pivotal principle for agricultural sustainability and managing soil fertility.

Organic fertilization is an important soil amendments used in agricultural systems to improve the soil's microbial diversity, fertility and water- holding capacity [3]. The nutrients required by the crop can be provided by organic amendment such as green manure in combination with mineral fertilizer which can improve soil fertility and limit the deleterious ecological impacts of intensive agriculture. Green manures are crops primarily grown as a nutrient source for subsequent crops. It is an agricultural management practice which maintains soil organic content by microbial mediated green manure decomposition and nutrient release. Such agricultural practices have significant implications on the microbial population, their functional activity and soil fertility.

The soil microorganisms are very sensitive to the changes in agricultural practices such as application of green manure. Fertilizers (organic and inorganic) can have direct impact on the growth of microbial population such as methanotrophs (specifically present in rice field) as whole by supplying nutrients which may affect their composition in soil. Methanotrophs occur at the aerobic-anaerobic interface, for example, at the surface of flooded paddy soil, on the rhizosphere and rhizoplane of rice plant roots. In rice rhizosphere, the thin layer of soil that is immediately adjacent to plant roots appears to be a very heterogeneous habitat for methanotrophs. Methane-oxidizing bacteria are obligate aerobic bacteria that can utilize methane as their sole source of carbon and energy for growth [4]. Hence, they are considered to be important regulators of atmospheric methane fluxes in nature. Flooded rice fields are one of the major sources for atmospheric methane (CH_4) which are responsible for the global anthropogenic emissions of methane gas [5]. Before releasing into the atmosphere, the produced CH_4 is subject to oxidation by methane-oxidizing bacteria (methanotroph). So, the population size of methanotrophic bacteria (MB) may be one of the important factors that govern the extent of CH_4 consumption in paddy fields. However, there is relatively little information present on the effects of applying green manures along with chemical fertilizers on methanotrophic population in paddy soil.

It is important to understand how nutrient resource inputs regulate the methanotrophic microorganisms, soil enzyme activity and nutrient levels in the soil. Monitoring and measuring soil enzymatic activities provide relevant indicators of soil functioning as well as soil functional biodiversity [6]. Soil functions are maintained by microbial flora present in soil which stimulates the production of extra- or intracellular enzymes and thereby have some impact on biogeochemical cycles. Thus, these relationships affect the economic justifiability of the use of different types, combinations and rates of fertilizers. The present paper thus addresses the effects of green manure with different combinations of plant density on the relationships between enzyme activity and soil nutrient level with respect to their impact on methanotrophic bacteria in rice crop.

2. MATERIALS AND METHODS

2.1 Experimental Design and Soil Sample Collection

A long term experiment since 2001 was established in a randomized block design (in triplicate) in research fields of Department of Agronomy, PAU, Ludhiana. Five replicates of rhizospheric soil samples were collected from each treatment plot. The rhizospheric soil is the soil associated with roots of the rice plants. The experiment consisted of different combinations of treatments (Table 1).

Sunn hemp (*Crotolaria juncea*) was used as green manure crop in the experiment. The source of nitrogen (N) was urea (46% nitrogen), phosphorus(P) was diammonium phosphate (18% N and 46% P) and potassium (K) was muriate of potash (60% K). The N was applied in three splits that is 1/3 at time of puddling; 1/3 N was applied 3 weeks after transplanting and remaining 1/3 N six weeks after transplanting. The P and K were applied at the time of transplanting. Treatment T1 which is a farmer's practice is considered as control treatment.

2.2 Enumeration of Methanotrophic Population

Soil samples were taken from rhizospheric soil of rice grown with different treatments under field conditions. Five soil samples were randomly taken from different areas of the same treatment field. These soil samples were mixed together to get one representative sample. Enumeration of methanotrophs was done on NMS medium [7] sterilized in an autoclave at 15 psi pressure and 121℃ temperature for 20 minutes. Enumeration of methanotrophic population was done by serial dilution spread plating technique and petriplates were incubated at 30℃ in air-tight chambers charged with (40%) methane in air in an inverted position [8]. After incubation, the number of colonies appearing on dilution plates were counted, averaged and multiplied by the dilution factor to find the number of cells per gram of soil sample.

2.3 Soil Enzyme Activity Assay

Soil enzymatic activities were analyzed from the same soil samples collected at different time intervals (0, 45, 90 DAT and at harvest). Alkaline phosphatase enzyme activity was determined by method of Bessey et al. [9]. This was a rapid method which employs β nitrophenyl phosphate that allows an instantaneous colour development at high pH. Dehydrogenase activity was assayed by method of Mersi and Schinner [10]. In this method (β-iodophenyl)-3-p(nitrophenyl)-5 phenyl tetrazolium chloride (2-INT) was used as substrate that get reduced and measured spectrophotometrically at 464 nm. Urease activity was estimated by measuring amount of urea remaining in soil solution [11] at 0, 45, 90 DAT and at harvest (120 DAT) of crop.

2.4 Estimation of Soil Nutrient Status

Soil samples was analyzed for soil organic carbon (OC) content by rapid titration method [12], soil nitrogen by modified alkaline potassium permagnate method [13], soil phosphorous by 0.5N sodium bicarbonate extactable P by olsen's method [14] and soil potassium by ammonium acetate extractable K [15] at 0, 45, 90 DAT and at harvest (120 DAT) of crop.

2.5 Statistical Analysis

The results were expressed as mean ± standard variation of three replicates. Data were subjected to correlation analysis by SPSS 16.0 software, analysis of variance (ANOVA) and difference between treatments were analysed by Duncun's test using SPSSv16.0 software.

Table 1. Different combinations of treatments

	Treatments
T1	22 plants /m^2 + 180 N/ha (farmer's practice)
T2	Recommended dose of NPK (120-30-30) kg/ha + 33 plants/m^2
T3	Green manure (15 t/ha) +33 plants/m^2+recommended NPK
T4	Green manure+ 44 plants/m^2 + recommended NPK
T5	Fertilizer on soil test basis (25% extra N, recommended dose of P & K) + 33 plants /m^2

3. RESULTS AND DISCUSSION

3.1 Effect of Green Manure and Plant Density Variation on Methanotrophic Population, Soil Enzyme Activities and Soil Fertility Status

Methane oxidizing bacteria (Methanotrophs) are associated with roots of rice plant [16]. They oxidize CH_4 with molecular O_2 and use it as sole source of carbon and energy. In this experiment, methanotrophic population was enumerated from rhizospheric soil of rice crop at different time interval. Methanotrophic population was observed to be significantly higher at 90 DAT than the methanotrophic population observed at 0, 45 DAT and at time of harvesting. The significantly higher methanotrophic count (176 × 10^4 cfu/g of soil) was observed at 90 DAT in treatment having green manure (15 t/ha) + 44 plants/m^2 + recommended NPK over methanotrophic population (169× 10^4 cfu/g of soil) in treatment T3 (green manure (15 t/ha) + 33 plants /m^2 + recommended dose of NPK fertilizer) (Table1). Statistical analysis revealed that increase in plant density and application of green manure led to significant increase in methanotrophic population as compared to methanotrophic population in farmer's practice (22 plants/m^2 + 180 N/ha) treatment i.e. 78×10^4 cfu/g of soil. Incorporation of organic manure enhanced the abundance of methanotrophic organisms in alkaline paddy soil. Present study revealed that the methanotrophic population in all treatments was reduced at 45 DAT as compared to population at 90 DAT. It might be due to the reason that saturation of soil with irrigation and heavy rainfall in paddy soil created stressful anoxic conditions. The results were in accordance with Dubey and Singh [17] that, the limited O_2 availability had major impact on growth, multiplication and survival of methanotrophs. Dubey [18] showed that methanotrophic population decreased gradually during the flooding of rice soil which was lead to increase in methane oxidation (dryland 64-86%; flooded rice soil 46 to 64%) as compared to bulk and bare soils.

Soil enzymatic activity was significantly influenced by green manure application and variation in plant density. Soil enzymatic activities increased significantly up to 45 DAT and then decreased till crop harvest (Table 2). The study was in accordance to Haung [19] that when the rice crop was at the most flourished stage then the soil enzymatic activities were at strongest stage, owing to the fact that the rice roots excreted enormous amount of organic acid and carbohydrate, which stimulated the correlative soil enzymatic activities. The highest dehydrogenase activity (50 µg TPF/g of soil/hour) was observed in treatment having green manure (15 t/ha) + 33 plants /m^2 + recommended dose of NPK fertilizer at 45 DAT. Increase in the dehydrogenase activity might be due to the flooded condition in rice field. Similar results were obtained by Gu et al. [20] which reported higher dehydrogenase level (even by 90%) in flooded soil, rather than in non-flooded conditions. Statistical analysis revealed that enzyme activity was positively correlated with application of green manure. The present study was in accordance to the Sriramachandrasekharan and Ravichandran [21] that different green manures caused about 40 per cent increase in dehydrogenase activity, while nitrogen fertilizer alone caused only 9.3% increase in dehydrogenase activity over control. Maximum urease activity (855 µg/g of soil/hour) was found in the treatment having green manure (15 t/ha) + 33 plants /m^2 + recommended dose of NPK fertilizer at 45 DAT. Soil urease activity was declined in prophase and ascended in anaphase and reached peak value while entering the growth peak period, similar results were reported by Sriramachandrasekharan and Ravichandran [21]. On the other hand, highest alkaline phosphatase activity (21.9 µg pNP/g of soil/hour) was observed in treatment with green manure (15 t/ha) + 44 plants/m^2 + recommended NPK at 45 DAT. The results showed that increase in plant density significantly affect the alkaline phosphatase activity. The decomposition of green manure resulted in the release of higher quantity of native phosphates and increased availability of applied phosphates. Roldan et al. [22] reported that higher amount of acid phosphatase activity in soil was observed with addition of crop residue as compared to the control, which occurred due to enhanced availability of phosphorus with incorporation of green manure.

Significant increase in soil nutrients were observed in all treatments except in farmer's practice at each time interval. Significantly higher soil phosphorus (35.8 kg/ha) and soil potassium (212.0 kg ha^{-1}) were observed in treatment having green manure+ 44 plants/m^2 + recommended NPK at 45 DAT (Table 1). This might be due to application of green manure which increased the available nutrients in soil due to its extensive root system, thus improved the soil physical condition along with dissolution of native nutrients. Several workers had reported a solubilizing effect of decomposing organic matter on P, K and trace elements. Sultani [23] observed that green manure *sesbania* on the average increased soil organic matter by 20%, N by 23%, P by 71% and K by 29%. Ahmad [24] revealed that green manure legume contributed substantial amount of K, some P and micronutrients in addition to N. Among nutrients, K was present in greatest concentration in the green manure legumes. However, soil nitrogen (206.9 kg/ha) was significantly higher in treatment having green manure (15 t/ha) + 33 plants /m^2+ recommended dose of NPK fertilizer at 45 DAT. This might be due to application legume green manure which benefits the soil nitrogen dynamics by recovering mineral N in soil and by fixing N from atmosphere. Legume green manuring directly served as N source in addition to increased mineralization of the native soil N pool. Organic carbon content in treatments having incorporation of green manure was significantly higher over the control at each time intervals. Organic carbon (0.59%) observed in treatment having green manure (15t/ha) + 44 plants/m^2 + recommended NPK dose was significantly higher at the harvest (Table 2). Organic carbon increased significantly from transplanting to harvesting of crop. It might be due to increased microbial activity which caused decomposition of green manure, resulting in increased organic carbon. This study was in accordance to Zhang et al. [25] that by adding amelioration organic manure or green manure significantly increased organic C storage in soil. Hababi et al. [26] revealed that different plants planted as green manure has significantly influenced the soil organic carbon.

3.2 Correlation Analysis between Methanotrophic Population, Soil Enzyme Activity and Soil Nutrient Status

Methanotrophs are the primary biological mechanism for mitigating the release of methane by consuming it as a source of carbon and energy. These microorganisms were significantly influenced by the soil nutrient level in the rice rhizosphere. The correlation analysis showed that increase in nutrient level in soil positively influenced the methanotrophic population. There was a positive correlation (r = 0.570 at P=0.05; r= 0.782, 0.822, 0.854 at P=0.01) between the available nitrogen and methanotrophic population at 0, 45, 90 DAT and at harvest of crop respectively. Statistical analysis also showed a significant positive correlation between the methanotrophic population with available phosphorus and potassium content (at P=0.01 level of significance). Although, available potassium and organic carbon were not increased significantly within treatment but both the parameters had significant positive correlation with methanotrophic population at all time intervals (Table 3). The results suggested that increase in application of nitrogen fertilizer caused significant increase in methanotrophic population. Similar reports was given by Bodelier et al. [27] that N fertilization increased methane oxidation in densely rooted rice soil, which was stimulated by increased nitrogen availability due to un-quantified nitrogen limitation of methanotrophs. Zheng et al. [28] also reported that application of nitrogen and potassium together or the combination of nitrogen, phosphate, potassium along with crop residue stimulated the growth of methanotrophic communities. Result analysis suggested a positive relation between organic carbon and methanotrophic population. These results showed that incorporation of crop residues and other organic amendments enhanced the abundance of methanotrophic organisms in alkaline paddy soil which in turn increased the soil fertility.

Nutrient transformation process is necessary for plant growth promotion which was usually mediated by the enzymes present in the soil. In the present study, soil urease activity showed a variable correlation with phosphorus and potassium at different time intervals. Activity of urease enzyme showed a positive but non-significant correlation with phosphorus and available potassium content at 90 DAT and at harvest of crop. The urease has significant positive correlation with available nitrogen content at all time intervals (Table 4). The results indicated that there was a positive correlation of urease with different major nutrients in soil. The organic carbon level significantly correlated with the urease activity at 0 and 45 DAT which indicated that organic carbon maintains the microbial population thus the microbial assisted soil urease activity. This might be due to microbial decomposition of applied green manure which maintained the soil organic matter in soil. Urease activity of soils was correlated with their organic carbon and available N contents as it might be attributed to higher N content, faster decomposition and release of NH_4-N.

Table 2. Effect of green manure and plant density on enzyme activities, soil nutrient and on methanotrophic bacteria in soil in different treatments at different time interval in field condition

Treatments	DAT	Methanotrophic population (10^4 cfu/g of soil)	Soil Enzyme activity			Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Organic carbon (%)
			Urease (µg/g soil/hr)	Alkaline phosphatase (µg pNP/g soil/hr)	Dehydrogenase (µg TPF/g soil/hr)			
T1-	0	19±0.57e	375.0±1.00e	02.0±1.00e	04.0±1.0c	136.7±0 .10e	26.0±0.36e	0.30±0.001e
22 plants /m^2 + 180 N/ha	45	70±0.57e	665.0±1.00e	11.2±0.21e	15.3±0.1e	174.2± 0.31e	30.6±0.30e	0.38±0.002e
	90	78±0.58e	245.0±1.00e	09.2±0.31e	10.2±0.1d	155.2± 0.20e	25.4±0.25e	0.40±0.002e
	Harvest	35±0.58e	150.0±0.31e	05.8±0.43e	01.2±0.1e	1 48.2±0.20e	23.8±0.10d	0.43±0.001e
T2-	0	41±0.55c	480.0±1.0d	08.0±0.05c	10.0±0.57b	167.8±0 .31c	28.1±0.31c	0.34±0.001c
NPK (120-30-30) kg/ha + 33 plants/m^2	45	97±0.57c	745.0±1.0d	15.8±0.31d	25.0±1.00d	187.3± 0.32c	32.4±0.15c	0.40±0.001c
	90	146±1.0c	340.0±1.0c	13.4±0.31d	12.5±0.15c	158.7± 0.42d	28.0±0.38c	0.43±0.001d
	Harvest	48±0.0c	185.0±1.0c	09.2±0.31d	02.5±0.10d	15 2.6±0.15c	24.2±0.25c	0.49±0.001c
T3-	0	46±1.15b	655.0±1.0a	10.6±0.20b	12.5±0.1a	188.4±0. 15a	30.4±0.15b	0.36±0.002b
Green manure (15 t/ha) +33 plants/m^2+recommended NPK	45	105±1.0b	855.0±1.0a	18.8±0.31b	50.0±1.0a	206.9±0 .42a	34.6±0.10b	0.42±0.001b
	90	169±2.52b	460.0±1.0a	17.0±0.31b	35.0±1.0a	175.6± 0.21a	29.1±0.32b	0.48±0.001b
	Harvest	83±2.64b	235.0±1.0a	13.6±0.35b	27.0±1.0a	17 2.8±0.28a	25.2±0.20b	0.55±0.002b
T4-	0	102±1.0a	610.0±1.0b	21.0±0.50a	15.0±1.0a	176.8±0. 15b	31.6±0.20a	0.38±0.002a
Green manure+ 44 plants/m^2 + recommended NPK	45	137±2.0a	810.0±1.0b	21.9±0.32a	37.5±0.5b	199.8±0 .38b	35.8±0.21a	0.43±0.007a
	90	176±2.0a	305.0±1.0d	21.2±0.17a	27.0±1.0b	169.8±0 .25b	30.3±0.30a	0.51±0.003a
	Harvest	108±1.0a	167.5±0.1d	18.0±0.46a	12.0±1.0b	16 4.2±0.25b	26.4±0.1a	0.59±0.002a
T5-	0	23±1.0d	590.0±1.0c	05.0±0.34d	05.0±1.0c	163.7±0.2 1d	27.9±0.21d	0.32±0.002d
25% extra N, recommended dose of P & K) + 33 plants /m^2	45	83±1.0d	770.0±1.0c	16.0±0.80c	25.0±1.0c	182.4±0. 10d	31.8±0.15d	0.39±0.002d
	90	97±1.0d	360.0±1.0b	15.2±0.10c	13.0±1.0c	159.4±0. 10c	27.2±0.15d	0.45±0.001c
	Harvest	43±1.0d	200.0±1.0b	11.6±0.15c	04.2±1.0c	150 .4±0.31d	24.6±0.20e	0.48±0.003d

Values with different letter(s) are significantly different at p =0.05

Table 3. Correlation analysis of methanotrophic population with soil enzymatic activities and soil nutrients observed at different time intervals

Methanotrophic population	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})	Organic carbon (%)
0 DAT	0.570*	0.887**	0.955**	0.899**
45 DAT	0.782**	0.951**	0.888**	0.918**
90 DAT	0.822**	0.958**	0.910**	0.819**
Harvest	0.854**	0.964**	0.991**	0.972**

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

Table 4. Correlation analysis of urease activity with soil available nutrients observed at different time intervals

Urease activity	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})	Organic carbon (%)
0 DAT	0.910**	0.790**	0.658**	0.755**
45 DAT	0.942**	0.847**	0.782**	0.856**
90 DAT	0.715**	0.446	0.426	0.450
Harvest	0.626*	0.269	0.286	0.365

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

Table 5. Correlation analysis of dehydrogenase activity with soil available nutrients observed at different time intervals

Dehydrogenase activity	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})	Organic carbon (%)
0 DAT	0.836**	0.940**	0.833**	0.964**
45 DAT	0.973**	0.843**	0.826**	0.859**
90 DAT	0.995**	0.757**	0.890**	0.821**
Harvest	0.970**	0.649**	0.793**	0.714**

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

Table 6. Correlation analysis of alkaline phosphatase activity with soil available nutrients observed at different time intervals

Alkaline phosphatase activity	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})	Organic carbon (%)
0 DAT	.650**	.929**	.969**	.934**
45 DAT	.853**	.954**	.899**	.935**
90 DAT	.787**	.911**	.915**	.991**
Harvest	.718**	.968**	.877**	.962**

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

Similar reports are given by Krishnamurthy et al. [29] that addition of organic manures increased the urease activity over mineral nitrogen to the significant extent. According to Xiefeng et al. [30] the urease activity increased with application of green manure for long term, which benefited the transformation of soil nitrogen.

The activity of dehydrogenase enzyme is basically representing the metabolically active microbial population. The correlation analysis of data revealed that the dehydrogenase was positively influenced by the increased nutrient and organic carbon levels in soil. It might be due to the enhance availability of nutrient to microbial population due to application of green manure in rice field. This was confirmed by correlation analysis that dehydrogenase activity of soil was positively correlated with organic carbon of soil at all time intervals. Dehydrogenase enzyme had a significant positive correlation with phosphorus and potassium content in soil (at P=0.01 level of significance) as shown in Table 5. The positive correlation of dehydrogenase with nutrient level might be due to application of green manure which increased soil aggregation and the water holding capacity of soil thus, increased dehydrogenase (water maintained the oxidative status) activity. Dinesh et al. [31] reported green manures recorded higher dehydrogenase activity as compared to chemical fertilizer, owing to increased availability of major nutrients and organic carbon which was reflected by higher microbial metabolism.

Alkaline phosphatase is an enzyme of great agronomic value because it hydrolyses organic phosphorus and transforms them into different forms of inorganic phosphorus that can be assimilated by plants. The correlation analysis showed that alkaline phosphatase activity was significantly related with available phosphorus (at P=0.01) indicating that enzyme actively dissolve the soil phosphorus (Table 6). The enzyme activity showed a significant positive correlation with available nitrogen and potassium which might be due to increased availability of nutrients to microbial population with green manure application thus enhanced enzyme activity. The addition of crop residue increase the phosphatase activity in soil which attributed to enhanced microbial activity with incorporation of green manure. A study done by Piotrowaska and Wilczewski [32] reported a significant positive correlation between phosphatase activity and available phosphorus in soil. The addition of organic substances to the soil served as a carbon source that enhanced microbial biomass and phosphatase activity, showed that these enzymes were of microbiological origin and crop growth stage significantly influenced soil enzyme activities [33].

4. CONCLUSION

It is concluded that application of green manure with increased plant density positively influence the soil enzymatic activities and hence soil nutrient status of soil. The green manure application increases the soil nutrient content which has significant positive correlation with methanotrophic population and enzymatic activities. So, this organic amendment maintains a sustainable agroecosystem with a lesser pollution potential due to increased population of methanotrophic bacteria and low dose of chemical fertilizer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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