Research Article

Effect of Long Term Incorporation of Organic and Inorganic Fertilizers on Phosphate Solubilizing Bacteria and Alkaline Phosphatase Activity

Jupinder Kaur* and S. K. Gosal

Department of Microbiology, Punjab Agricultural University, Ludhiana-141 004, Punjab, India

Abstract

Soil phosphate solubilizing bacteria and activity of soil phosphatase enzyme strongly control the biotic pathways of phosphorus which is an essential element for life and is often limiting in terrestrial ecosystems. Information on the role of soil phosphate solubilizing bacteria and phosphatase activity is fundamental for understanding soil phosphorous dynamics but remains poorly understood. A study was conducted in a long-term field experiment started in 2005 at the experimental area of Department of Soil Science, Punjab Agricultural University, Ludhiana to explore the long-term effects of organic and inorganic fertilizers on the population of soil phosphate solubilizing bacteria and alkaline phosphatase activity in rhizosphere of rice crop. Soil phosphate solubilizing bacteria and phosphatase activity exhibited significant changes in response to long-term fertilization. The highest population of phosphate solubilizing bacteria (190×10^2 cfu/g soil) was recorded during flowering stage of rice crop in treatment T11 with 7.5 ton rice straw/ha + 120 Kg nitrogen/ha, whereas, highest activity of soil alkaline phosphatase activity (25.9 µg PNP/hour/g soil) was observed in treatment T16 with 10 ton rice straw/ha and 150 Kg nitrogen/ha. The results suggested that long term application of organic and inorganic fertilizers may maintain soil quality and productive capacity by recycling of phosphorous.

Keywords: Inorganic fertilizers, organic fertilization, phosphate solubilizing bacteria, soil alkaline phosphatase activity, rice crop

***Correspondence**
Author: Jupinder Kaur
Email: jupindercheema13@gmail.com

Introduction

Phosphorus (P) is an essential element for energy transport, cellular structures and is thus necessary for life. The weathering of parental material and the input from atmospheric dust deposition are the two main P sources for ecosystem. Apart from essential, soil phosphorous is also a limiting element for plant growth and its turnover in soil play an important role in determining the overall biological availability of P, which in turn influences ecosystem productivity [1]. Because organic P must be hydrolyzed by phosphate solubilizing bacteria (PSB) and phosphatases enzymes to be available for plants, thus, the activities of these bacteria and enzymes (acid phosphomonoesterase, alkaline phosphomonoesterase etc.) play a major role in organic P cycling [2]. Soil phosphorous solubilizing bacteria are the bacteria that convert unavailable forms of phosphorous to plant utilizable form by array of mechanisms like organic acid production, chelation etc. whereas, phosphatase enzymes are produced by bacteria, fungi and plant roots. These enzymes cleave a phosphate group from its substrates; transform complex and sometime unavailable forms of organic P into assimilable phosphate. The activity of soil P-solubilizing bacteria and phosphatase production depends on a combination of P demand from plants and microbes, available organic P substrate and P limitation of the soil. However, the variation of soil phosphate solubilizing bacteria and phosphatase activities under different fertilizer regimes remain poorly understood despite extensive investigation [3].

The turnover of soil organic P is influenced by its chemical nature and associated reactivity. Many studies have shown that the amounts and forms of organic P in soil are influenced by environment conditions as well as land use and management including nutrient availability and organic matter nutrient inputs [1]. For example, soil organic P may decrease significantly in response to long term cultivation and crop removal [4]. Long term atmospheric nitrogen (N) deposition and P fertilizer inputs, both facilitated more P stored in organic forms [5]. Furthermore, the transformation and recycling of these P forms in the soil environment is regulated by population of soil phosphate solubilizing bacteria and phosphatase activities [6]. But, the population of soil phosphate solubilizing bacteria and activity of soil phosphatase enzyme is sensitive to farming practices practice like type of fertilization [7]. For example, several studies have shown that the activities of phosphatase in natural forest or grassland were higher than those in cultivated soil [8]. Application of inorganic P can inhibit phosphatase activities in soil through repressing the synthesis of phosphatase [2]. However, the effect of different fertilizer regimes (organic and inorganic fertilization) on function of soil phosphate solubilizing bacteria and phosphatase activities still remains poorly understand. Organic and inorganic fertilizers not only serve to maintain or improve crop yields, but their application directly or indirectly cause changes in chemical, physical and biological properties of the soil. These changes, in the long-term, are believed to have significant influences on the different groups of bacteria, enzyme activities, quality and productive capacity of the soil [9]. However, available information is conflicting and uncertainties still remain about the long-term influences of different fertilizers regimes on soil phosphate solubilizing bacteria and phosphatase activities. These conflicting reports emphasize the need for research on long-term effects of organic and inorganic fertilizer application on bacteria and enzymes linked with various biogeochemical cycles [10]. As the effects of management practices are best evaluated using long-term experiments [11] so, a long term field experiment that has been ongoing at the Punjab Agricultural University provided a unique opportunity for such a study. In the course of the experiment, investigations were conducted on soil phosphate solubilizing bacteria and soil alkaline phosphatase activity of rice crop.

Material and Methods

Experimental design

A long-term field experiment (started in 2005) laid out in split plot design in triplicate at the experimental area of Department of Soil Science, Punjab Agricultural University, Ludhiana was used to explore the long-term effect of organic (rice straw-RS) and inorganic fertilization (nitrogen) on the population of soil phosphate solubilizing bacteria and alkaline phosphatase activity in rhizosphere of rice crop at different growth stages of rice crop. The rice crop (variety - PR 118) was raised by following the crop management practices recommended in Package of Practices, PAU, Ludhiana. A total of 16 different combinations of rice straw (0, 5 ton/ha, 7.5 ton/ha and 10 ton/ha) and urea fertilizer (0, 90 Kg/ha, 120 Kg/ha and 150 Kg/ha) were made which are listed in **Table 1**.

Table 1 Different treatments

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

RS: Rice straw and N: Nitrogen

Soil sampling

Soil samples were collected from rhizospheric soil of rice crop at different growth stages of rice crop i.e. transplanting, maximum tillering, flowering and harvesting stage. Plants were excavated 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 the roots and kept as rhizospheric soil. The five rhizospheric samples from each treatment were combined, passed through 2-mm sieve and stored at 4° C until required for analysis.

Enumeration of soil phosphate solubilizing bacteria and assay of soil alkaline phosphatase activity

Enumeration of soil phosphate solubilizing bacteria was done on Pikovskaya's medium using serial dilution spread plate technique. The bacterial colonies with clear zone (due to solubilization of organic phosphorous) around them were counted and expressed as cfu/ g of soil. Alkaline phosphatase activity was assayed with the method of Tabatabai and Bremner [12] using para nitro phenylphosphate (PNPP) as substrate and the rate of release of para nitro-phenol (PNP) was determined.

Statistical analysis

To determine the effect of different fertilizer regime, stages of plant growth and their interaction on soil PSB population and phosphatase activity, analysis of variance (ANOVA) was used with a probability P=0.05.

Results and Discussion

PSB population

The results indicated significant difference (CD@5%: Environment: 0.430; Treatments: 0.165; Interactions: 0.662) in population of soil phosphate solubilizing bacteria among different treatments at each time interval (transplanting stage, tillering stage, flowering stage and harvesting stage). Maximum PSB population (190 $\times 10^2$ cfu g^{-1} of soil) was observed in treatment T11 with 7.5 ton RS/ha + 120 Kg N/ha (**Figure 1**). The PSB population in treatments with integrated application of rice straw as organic amendment and inorganic fertilization was significantly higher than the population of these bacteria in treatments with solitary application of rice straw or inorganic nitrogen. This reflects the positive impact of balanced nutrient application on these bacteria. The population of PSB decreased during flooding conditions (tillering stage). This might be due to the fact that under flooded conditions, the redox potential of paddy is low and NO_3^-, Fe^{3+}, Mn^{4+}, and SO_4^{2-} are, respectively, reduced to NH_4^+, Fe^{2+}, Mn^{2+} and S^{2-}. Thus, flooding improves the availabilities of elements like P, K, Si, Mo, Cu, and Co [13]. So, P availability initially increased on flooding and population of phosphate solubilizing bacteria decrease. Maximum population of these bacteria was observed at the flowering stage time interval owing to establishment of the microflora whereas decrease in the population of phosphate solubilizing bacteria was observed as the crop proceeds towards maturity.

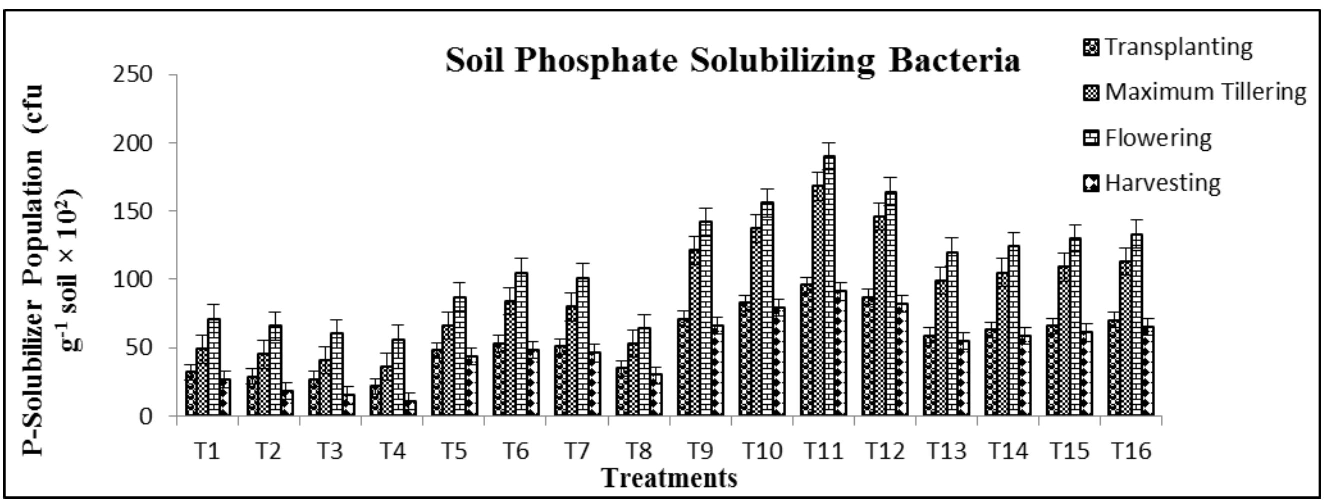

Figure 1 Effect of incorporation of rice straw and inorganic nitrogen fertilization on population of soil phosphate solubilizing bacteria at different growth stages of rice crop

Alkaline phosphatase

Alkaline phosphatase is an enzyme of great agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus that are assimilated by plants [14]. Data of alkaline phosphatase tended to be lowest in the control treatment T1 (0 ton RS/ha + 0 N Kg/ha) at all the growth stages of rice crop (before sowing, maximum tillering, flowering and at harvest of crop). The alkaline phosphatase activity was found to be significantly higher (CD@5%: Environment: 1.25; Treatments: 0.609; Interactions: 2.43) in treatments with integrated application of rice straw and inorganic fertilization relative to activity of this enzyme in treatments with solitary application of rice straw or inorganic nitrogen (**Figure 2**). Maximum activity of alkaline phosphatase

(25.9 µg pNP/hour/g soil) was observed in treatment T16 with 10 ton RS/ha + 150 kg N/ha at flowering stage. The results were in accordance with Balakrishnan [15] who ascertain that the phosphatase activity is highly correlated with soil microbial biomass. The provision of organic carbon and stimulation of microbial growth under the combined treatments (organic + inorganic) could have elevated the synthesis of phosphatase enzymes thereby contributing to the soil phosphatase pool. Therefore, the application of organic fertilizers increased nutrient turnover through both increased microbial biomass and activity [16]. The results of the present study showed that integrated application of organic and inorganic amendments could significantly improve soil biological activity while maintaining crop yield.

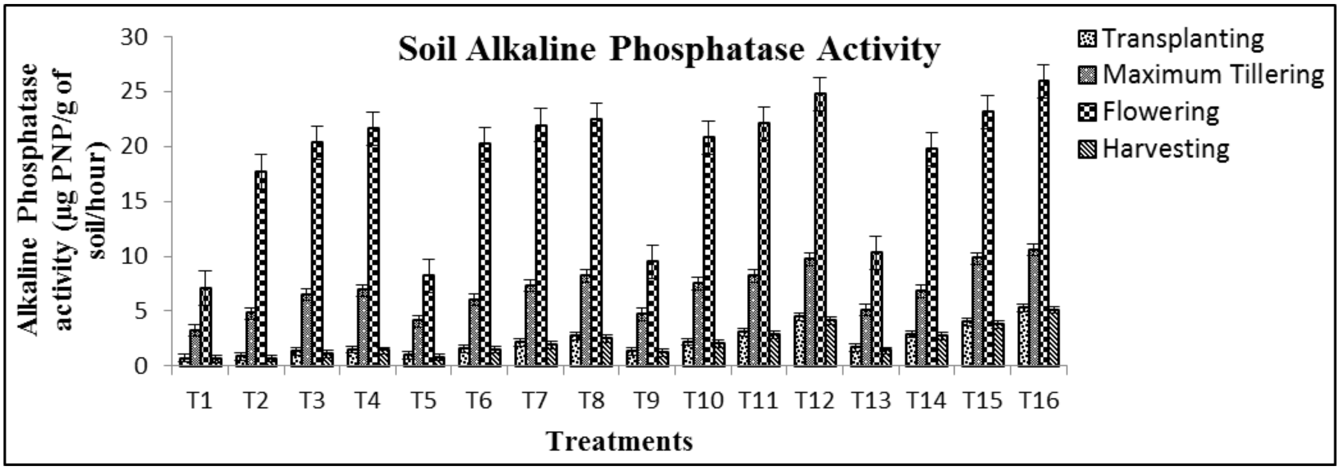

Figure 2 Effect of incorporation of rice straw and inorganic nitrogen fertilization on soil alkaline phosphatase activity at different growth stages in rice crop

Conclusion

The dynamics of soil phosphate solubilizing bacteria and the activity of soil phosphatase enzyme was significantly influenced by the crop growth stages and different fertilizer regimes. The integrated application of rice straw and inorganic nitrogen emerged out as the best treatment compared to mono application of either rice straw or inorganic nutrients. Application of organic amendments along with inorganic nutrient levels not only improved soil biological activities but also significantly contribute to nutrient buildup in soil. It also maintained a balanced enzymatic activity with a lesser pollution potential then high dose of nutritional levels.

Acknowledgements

The authors wish to acknowledge Maulana Azad National Fellowship for providing financial support to carry out this research work.

References

[1] L. M. Condron, B. L. Turner and B. J. Cade-Menun (2005) Chemistry and dynamics of soil organic phosphorus. In: J.T. Sims and A.N. Sharpley, editors, Phosphorus: Agriculture and the environment. Agron Monogr 46: 87-121.

[2] P. Nannipieri, L. Giagnoni, L. Landi and G. Renella (2011) Role of phosphatase enzyme in soil. In: Bunemann K E et al (ed) Phosphorus in action. Soil Biol 26:215-43.

[3] L. M. Condron and and H. Tiessen (2005) Interactions of organic phosphorus in terrestrial ecosystems. In: B. L. Turner, E. Frossard, D. S. Baldwin (Eds.). Organic phosphorus in the environment. Cabi Publ., Cambridge, MA. EUA. Pp 295-308.

[4] A. Tiessen, K. Prescha, A. Branscheid, N. Palacios, R. McKibbin, N.G. Halford and P. Geigenberger (2003) Evidence that SNF1-related kinase and hexokinase are involved in separate sugar-signaling pathways modulating post-translational redox activation of ADP-glucose pyrophosphorylase in potato tubers. Plant J 35:490-500.

[5] B. L. Turner and L. M. Condron (2013) Pedogenesis, nutrient dynamics, and ecosystem development: The legacy of T.W. Walker and J. K. Syers. Plant Soil 367:1–10.

[6] V. Acosta-Martínez and M. A. Tabatabai (2000) Enzyme Activities in a Limed Agricultural Soil. Biol. Fert. Soils 31:85-91.
[7] P. Nannipieri, E. Kandeler and P. Ruggiero (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick R (Eds) Enzymes Environ, 1-33. Marcell Dekker, New York.
[8] E. L. Balota, I. F. Yada, H. Amaral, A. S. Nakatani, R. P. Dick, and M. S. Coyne (2013) Long term land use influences soil microbial biomass P and S, phosphatase and arylsulfatase activities, and mineralization in a brazilian oxisol. Land Degrad Dev 25:397-406.
[9] D. F. Acton and Gregorich (1995) The health of our soils towards sustainable agriculture in Canada, CDR unit, Ottava.
[10] T. Dobbs and J. D. Smolik (1996). Productivity and profitability of conventional and alternative farming systems: a long- term on- farm paired comparison. Journal of Sustainable Agriculture 9:63-77.
[11] P. C. Nel, R.O. Barnard, R. E. Steynbergde, J. M. Beer and H. T. Groeneveld (1996) Trends in maize grain yields in a long-term fertilizer trial. Field Crops Res. 47:53-64
[12] M. A. Tabatabai and J. M. Bremner (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1:301–307.
[13] M. S. Fan, R. F. Jiang, F. S. Zhang, S. H. L u and X. J. Liu (2008) Nutrient management strategy of paddy rice-upland crop rotation system. Chinese Journal of Applied Ecology 19(2):424-432.
[14] F. T. Maestre, M. D. Puche, C. Guerrero and A. Escudero (2011) Shrub encroachment does not reduce the activity of some soil enzymes in Mediterranean semiarid grasslands. Soil Biol Biochem 43: 1746-49.
[15] N. Balakrishnan, R. Venkatesan, K. C. Ravindran (2007) The influence of halophytic compost, farmyard manure and phosphobacteria on soil microflora and enzyme activities. Department of Botany, Faculty of Science, Annamalai University.
[16] S. Saha, V. Prakash, S. Kundu, N. Kumar and B. L. Minna (2008) Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N–W Himalaya. Eur J Soil Biol 44: 309-15.

Publication History
Received 02nd Dec 2017
Revised 18th Dec 2017
Accepted 20th Dec 2017
Online 30th Dec 2017