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Soil Enzyme Activity as Influenced by Seasonal Rainfall and Crop Growth Stages under Long-Term Fertilization and Intensive Cropping with Hybrid Maize

Pragyan Paramita Rout[1*], N. Chandrasekaran[1] and Dhaneshwar Padhan[2]

[1]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore-641003, India.
[2]Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal-741235, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors PPR and NC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PPR managed the analyses of the study. Author DP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Soil biological health mediated by enzyme activity is an index for soil fertility and plays a significant role in sustainability of cropping system. A field experiment was conducted in 2013-14 under AICRP-LTFE to study the influence of seasonal rainfall and crop growth stages on soil enzyme activities under long-term fertilization. Ten treatments were imposed as follows: T_1 - 50 % NPK, T_2 - 100% NPK, T_3 - 150% NPK, T_4 - 100% NPK + hand weeding, T_5 -100% NPK + $ZnSO_4$, T_6 -100% NP, T_7 -100% N alone, T_8 -100% NPK + FYM, T_9 -100% NPK (-S) and T_{10} - Control. The occurrence of monsoon rainfall increased the soil urease and dehydrogenase enzyme activity whereas phosphatase activities recorded lower values. The results also indicated high influence of crop growth stages on enzyme activities which is well evidenced by high phosphatase activity, low

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**Corresponding author: E-mail: parimita.pragyan00@gmail.com;*

urease and dehydrogenase activity at harvest stage of hybrid maize. Among treatments, T_8 - 100% NPK + FYM at 10 t ha^{-1} recorded highest activities of these enzymes emphasising importance of integrated nutrient management in improving biochemical properties thereby sustaining soil health over long run.

Keywords: Monsoon rainfall; soil biological health; soil enzyme activity.

1. INTRODUCTION

Enzymes play key role in the cycling of nutrients in soil and their activity is considered as an indicator of soil fertility [1]. Microorganisms, active plant roots and dead microbial cells are the principal sources of soil enzymes [2]. Soil urease is an extracellular enzyme involved in the hydrolysis and transformation of urea fertilizers [3]. Soil phosphatase hydrolyses the ester bonds (C-O-P) in organic matter there by releasing inorganic P from organically bound P [4]. It has been reported that under phosphorus deficiency conditions, both plants and microorganisms release phosphatase enzymes into the soil which have potential to mobilize P reserve [5]. Similarly dehydrogenase enzyme oxidizes soil organic matter and is considered as an indicator of overall microbial activity in soil because it occur intracellularly in all living microbial cells and is linked with microbial oxido-reduction processes [6].

The activity of soil enzymes is greatly affected by various abiotic factors such as temperature, moisture, pH, and oxygen content [7]. It is reported that temperature and moisture influence enzyme activities indirectly through microbial growth and substrate availability [8] which can be well evidenced by the findings of [9] who stated that soil urease activity is higher in winter than in summer. Similarly soil enzyme activity also exhibits temporal variation as growth stages of crop proceeds. It is observed that higher activities of urease, phosphatase and catalase in soil during vigorous growth stage of cucumber while lower activity during the early and late growth stages [10]. Hence, as the seasonal changes and growth stages of crop affects soil enzyme activity, so quantifying such changes in enzyme activity provides an index for the nutrient transformation reactions in soil [11].

In the context of soil fertility management, long-term fertilizer experiments (LTFEs) are valuable assets for determining yield trends, changes in nutrient dynamics and balances, predicting soil carrying capacity, assessing soil quality and system sustainability [12]. Besides, soil enzyme activities are also influenced by fertilization, agricultural management practices such as crop rotation, amendments, tillage under intensive cropping system [13]. It has been reported that the activities of dehydrogenase, acid phosphatase and protease in soil is positively influenced by organic amendments *viz.,* vermicompost from biological sludge and stabilized dairy manure than inorganic amendments (ammonium nitrate) [14]. As reported by [15], phosphatase activity increased when glucose and nitrogen sources are added to the soil through organic source of amendments (vermicompost and stabilized manure) due to equilibrated balance between N and C. Study on soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems reported a 46% and 61% increase in acid and alkaline phosphatase activities respectively due to no tillage practices (NT) than conventional tillage (CT) under maize-wheat crop rotation due to the high biomass production of maize that would produce greater amounts of substrate for microbial growth and production of enzymes [16].

In contrast, information on the biological processes, such as soil enzymatic activities, which mediate nutrients cycling and influence their acquisition during active crop growth stages under LTFEs, are limited. Keeping this in mind, we tried to quantify the effect of long-term fertilization on seasonal and temporal variations in soil enzyme activity during monsoon fallow period followed by cropping period with hybrid maize.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted during 2013-14 as a part of an ongoing All India Coordinated Research Project (AICRP) on Long-term Fertilizer Experiment (LTFE) with finger millet-hybrid maize cropping sequence which was initiated in 1972 at Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu. The experimental research farm is situated at 11° North latitude, 77° East longitude and at an altitude of 426.7 meter above mean sea level

(MSL). In this experiment, finger millet crop was grown during kharif (June to September) followed by hybrid maize grown during rabi (December to May). In between finger millet and hybrid maize the experimental field was left fallow during October to November that receives rainfall due to North East monsoon. Hence, present investigation was undertaken to record the seasonal changes in soil enzyme activity during monsoon fallow period (after harvest of 100th finger millet crop) when rainfall occurs due to North East monsoon followed by temporal changes during cropping period with hybrid maize (101th crop).

2.2 Weather and Soil Type

During the study period (October 2013-May 2014), the experimental location recorded a maximum and minimum temperature of 17.2℃ and 34.5℃ respectively with a total rainfall of 8.1 mm. The soil of experimental site belongs to *Inceptisol* order, having calcareous mixed black soil with sandy clay loam texture and comes under Perianaickenpalayam series of *Vertic Ustropept*. The initial soil properties of experimental site have been presented in Table 1.

2.3 Treatment Details

Ten treatments were employed for the present study, each replicated four times in Randomized Complete Block Design (RCBD). The treatment details have been given in Table 2. The hybrid maize variety *viz.,* CO-6 was used as test crop. The recommended dose of N, P_2O_5 and K_2O based on initial soil test was 250:75:75 kg ha^{-1}. The sources of N, P and K used were urea, single super phosphate (SSP) and muriate of potash. In sulphur free treatment (T_9), diammonium phosphate (DAP) was used instead of SSP as a source of P. All the treatments except T_4 was sprayed with the pre emergence herbicide Atrazine WP at 500 g ha^{-1}at 3rd days after sowing (DAS) for controlling of weeds.

2.4 Soil Sampling and Biochemical Analysis

Composite surface (0-15 cm) soil samples (*i.e.,* 10 random core samples from each plot were thoroughly mixed together) were collected from each plot both during monsoon fallow (at three stages viz., Before Rain Fall (BRF), After Rain Fall (ARF) and End of Fallow Period (EFP) followed by cropping period (at three critical growth stages of hybrid maize *viz.,* Knee high, Tasselling, Milky and Harvest). The soil phosphatase, dehydrogenase and urease activities were determined by *p*-nitro phenol method [17], TTZ (Tri Phenyl Tetrazolium Chloride) reduction method [18] and THAM (Tris-Hydroxymethyl Amino Methane buffer) incubation followed by distillation method [19] respectively. All other soil parameters were analysed following standard protocols.

2.5 Statistical Analysis

The data on analysis of soil and plant samples, dry matter production, yield, nutrient uptake and content of hybrid maize were subjected to analysis of variance (ANOVA) to find out the magnitude of treatment effect on the various parameters. The correlation statistics [20] was also computed to elucidate and establish possible relationship among the soil and plant characteristics in relation to hybrid maize performance. Least significant difference (LSD at $P < 0.05$) was used to determine whether means differed significantly. For statistical analysis of data, Microsoft Excel (Microsoft Corporation, USA) and Agres statistical software were used.

Table 1. Initial properties of the experimental soil

Sl. no.	Properties	Initial (1972)
1.	Textural class	Sandy clay loam
2.	pH	8.20
3.	Electrical conductivity (dSm^{-1})	0.20
4.	Cation exchange capacity (cmol (p^+) kg^{-1})	25.2
5.	Organic carbon (g kg^{-1})	3.0
6.	Available nitrogen (kg ha^{-1})	178.0
7.	Available phosphorus (kg ha^{-1})	11.0
8.	Available potassium (kg ha^{-1})	810.0
9.	Available Zn (mg kg^{-1})	2.58
10.	Available Mn (mg kg^{-1})	2.74
11.	Available Cu (mg kg^{-1})	4.20
12.	Available Fe (mg kg^{-1})	2.74

Table 2. Details of experimental treatments

Treatments	Details
T_1	50% NPK
T_2	100% NPK
T_3	150% NPK
T_4	100% NPK + Hand Weeding (HW)
T_5	100% NPK + $ZnSO_4$ at 25 kg ha^{-1}
T_6	100% NP
T_7	100% N
T_8	100% NPK + FYM at 10 t ha^{-1}
T_9	100% NPK (Sulphur free source of fertilizer)
T_{10}	Control (Unmanured and unfertilized)

3. RESULTS AND DISCUSSION

3.1 Phosphatase Activity

The term phosphatase has been used to describe a broad group of enzymes that hydrolyse C–O–P ester bonds in organic phosphorus compounds *viz.,* phospholipids, inositol phosphates and pyrophosphates thereby convert them into inorganic P compounds in soil. The activity of phosphatases is important in studying the P cycle as it provides a route for P mineralization and plant uptake of available P. Hence, the soil phosphatase activity is highly correlated with the magnitude soil available P [21]. The activity of soil phosphatase was evaluated in terms of µg *p*-nitro phenol released per gram of soil per hour.

3.1.1 Acid phosphatase activity

The results obtained on the activity of acid phosphatase (Fig. 1) during monsoon fallow period showed that before rainfall stage (BRF), its activity was higher ranging from 24.6 to 42.3 µg *p*-nitro phenol released g^{-1} soil hr^{-1} followed by a decline after rainfall stage (ARF). The lowering in acid phosphatase activity during monsoon fallow period than cropping period coincides with the findings of [22] showing significant seasonal effects on soil phosphatase activity and reported a sharp increase in acid phosphatase due to wheat cultivation compared to fallow field which indicates the strong rhizosphere effects on phosphatase activity.

The acid phosphatase activity was also found to be influenced by the growth stages of hybrid maize. The increase in acid phosphatase activity with the age of hybrid maize may be attributed to the development of root system with age and increase in total root surface area [23] followed by a decrease in activity at harvest stage. Though soil acid phosphatase activity was found to be high during early growth period of hybrid maize, but a reverse trend was observed in case of soil available P (with mean available P of 14.82, 13.0, 12.02 and 11.52 kg ha^{-1} at knee high, tasselling and milky stage respectively) reason being high uptake of P by hybrid maize during those period.

Among treatments imposed, markedly higher acid phosphatase activity was observed in control (T_{10}) irrespective of growth stages of hybrid maize. Such rise in activity might be caused due to the P stressed condition that had induced plant roots and microorganisms to release phosphatase enzyme to mobilize the native soil P reserve [5]. With increased soil acid phosphatase activity, it is obvious to record high available soil P in control. But on contrary, the available soil P status was found to be very low in control plots which might be due to uptake of soil available P by crop without external application of P fertilizers on long-term basis.

The profound influence of organic manures on acid phosphatase activity was evident from increased activity observed in the treatment receiving FYM at 10 t ha^{-1} along with 100% NPK (T_8). Such stimulation in activity upon addition of organic manure may largely be due to increased microbial population, which has caused a build-up of enzyme level in soil [24]. Furthermore, acid phosphatase activity was also found to decrease in the treatment receiving supplemental addition of $ZnSO_4$ at 25 kg ha^{-1} along with 100% NPK (T_8) which is in line with findings suggesting inhibitory effect of cations (Zn^{2+}) on acid phosphatase activity [25].

Similarly its activity was found to be low in treatment receiving 100% NP (T_6) when compared to treatment T_7 *viz.,* 100% N. Such decline might be due to the exclusion of fertilizer P application that in turn would have triggered the release of acid phosphatase in soil under P deficient conditions [26].

3.1.2 Alkaline phosphatase activity

The results on alkaline phosphatase activity (Fig. 2) showed that its activity was much higher than acid phosphatase, irrespective of the treatments imposed, which might be due to the alkaline reaction of the soil (pH=8.20). This is in consistent with earlier findings that phosphatase activity is strongly influenced by soil pH [21]. Initially at before rain fall (BRF) stage, the alkaline phosphatase activity ranged from 24.6 to 42.3 µg *p*-nitro phenol released g^{-1} soil hr^{-1}. The reduced alkaline phosphatase activity in monsoon fallow period was similar to that of acid phosphatase. This may be attributed to the increased moisture conditions that prevailed after rainfall leading to enhanced organic matter decomposition resulting in improved available P in soil which caused a reduction in alkaline phosphatase activity [27]. However, among the various stages compared, higher values were recorded at the harvest stage of maize irrespective of the treatments.

Among treatments, alkaline phosphatase activity was found to increase with application of 100% NPK fertilizers and FYM. This may be due to the fact that in general, the enzyme activity in the soil is closely related to the organic matter build-up [28]. A proportionate increase in the alkaline phosphatase activity was observed following the additions of nutrients with exception of the super optimal level of fertilizers (150% NPK) indicating the sensitivities of alkaline phosphatase to nutrient additions [29].

3.2 Dehydrogenase Activity

The activity of soil dehydrogenase (Table 2) was assessed in terms of µg tri phenyl formazone (TPF) released per gram soil per day. At before rain fall (BRF) stage, dehydrogenase activity ranged from 3.5 to 6.7 µg g^{-1} soil day^{-1}. Unlike phosphatase activity, dehydrogenase activity increased irrespective of treatments after monsoon rainfall (ARF). This may be reasoned to increased soil moisture after rainfall which favours the microbial population and decomposition of organic matter, resulting in increased activity of dehydrogenase [30]. At the end of monsoon fallow period (EFP), a sharp decline in dehydrogenase activity was recorded.

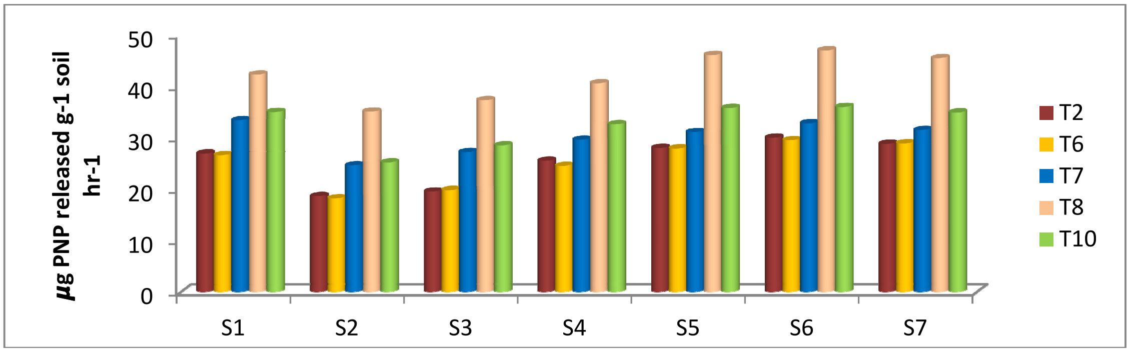

Fig. 1. Effect of long-term fertilization on seasonal and temporal changes in soil acid phosphatase activity (µg *p*-nitro phenol released g^{-1} soil hr^{-1})

S_1-before rain fall, S_2-after rain fall, S_3-end of fallow period, S_4-knee high, S_5-tasseling, S_6-milky, S_7-harvest

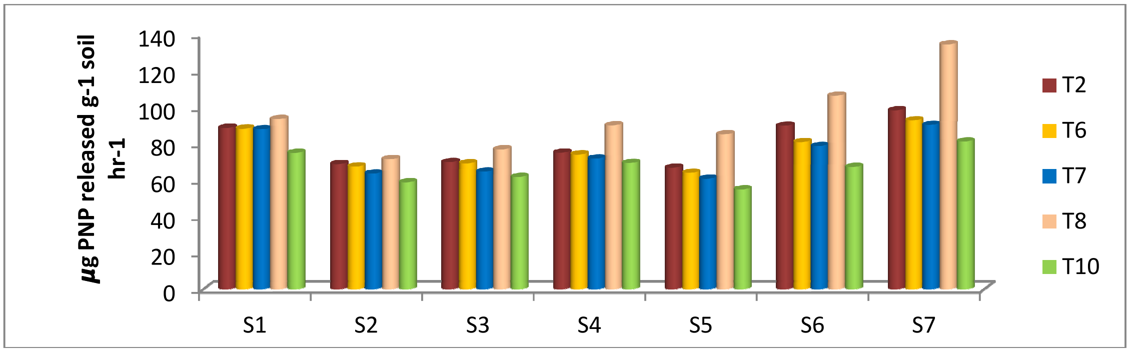

Fig. 2. Effect of long-term fertilization on seasonal and temporal changes in soil alkaline phosphatase activity (µg *p*-nitro phenol released g^{-1} soil hr^{-1})

S_1-before rain fall, S_2-after rain fall, S_3-end of fallow period, S_4-knee high, S_5-tasseling, S_6-milky, S_7-harvest

The dehydrogenase activity was found to be strongly affected by the crop growth stages. Among all growth stages of hybrid maize compared, knee high stage (S_4) showed the highest dehydrogenase activity and lower values were recorded during milky and harvest stage irrespective of the treatments which was consistent with the findings that reported temporal fluctuations in the activity of dehydrogenase at different growth stages of two rice varieties and found higher activities at seedling stage than tillering but decreased as rice crop matures [31].

A notable observation in the study was that dehydrogenase activity was poorly influenced by mineral fertilization. The result was consistent with the studies of [32]. The findings also suggested that exclusion of S from fertilizer schedule *viz.,* T_9-100% NPK (S free) caused a drastic reduction in dehydrogenase activity. According to [33], dehydrogenase activity is influenced more by the quality than by the quantity of organic matter incorporated into soil. Thus, the stronger effects of sulphur on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms.

A significant increase in dehydrogenase activity was evident due to combined application of FYM along with NPK fertilizers. The overall dehydrogenase activity ranged from 2.7 to 8.1 µg g^{-1} soil day^{-1}. The high organic matter levels in the FYM supplied treatment (T_8) might have provided a more favourable environment for the accumulation of enzymes in the soil matrix, since organic constituents are thought to be important in forming stable complexes with free enzymes. This was in line with the finding reported that dehydrogenase activity was increased by FYM applications as compared to straw treatments [34].

3.3 Urease Activity

The activity of soil urease (Table 4) was assessed in terms of µg NH_4^+ released g^{-1} soil hr^{-1}. With respect to seasonal change during monsoon fallow period, soil urease activity exhibited a similar trend as that of dehydrogenase recording higher activity after ARF stage followed by slight decline at the end of follow period (EFP). These results corroborates with the findings of [35] who reported that urease activity increased with increase in soil moisture content up to field capacity and remained constant with further increase in moisture content.

Higher urease activity was observed during the active crop growth of maize. Such results coincide with the findings of [36] who indicated that the development of crop growth may have greater impact on urease activity. The results also revealed significantly higher urease activity in plots receiving combined application of 100% NPK+FYM (T_8). The results were further confirmed by the findings of [37] who reported 9-12 increase in urease activity in plots receiving both compost and inorganic fertilizer as compared to control. Application of sub (50% NPK) and super optimal (150% NPK) levels of mineral N fertilizers caused a drastic reduction urease activity stating that its activity is mainly influenced by application of N [26].

Table 3. Effect of long-term fertilization on seasonal and temporal changes in soil dehydrogenase activity (µg TPF, Tri Phenyl Formazan released g^{-1} soil day^{-1})

Treatments	Monsoon fallow period			Cropping period			
	BRF	ARF	EFP	Knee high	Tasseling	Milky	Harvest
T_1	4.8	5.5	5.3	4.8	4.0	4.3	3.8
T_2	4.6	5.7	5.4	4.7	4.5	4.5	3.7
T_3	5.1	6.0	5.7	5.2	4.8	5.1	4.1
T_4	4.6	5.3	5.1	4.7	4.4	4.6	3.8
T_5	4.0	5.1	4.9	4.3	4.0	4.2	4.0
T_6	4.1	5.6	5.4	5.0	4.7	4.9	4.1
T_7	4.4	5.6	5.2	4.8	4.4	4.6	3.9
T_8	6.7	8.1	7.2	7.0	6.6	6.9	6.3
T_9	3.8	4.2	3.9	3.7	3.5	3.9	3.6
T_{10}	3.5	3.7	3.6	3.3	3.0	3.4	2.7
SEd	0.36	0.20	0.22	0.21	0.26	0.22	0.19
CD (P=0.05)	0.74	0.41	0.45	0.44	0.52	0.44	0.39

BRF: Before rain fall, ARF: After rain fall, EFP: End of fallow period

Table 4. Effect of long-term fertilization on seasonal and temporal changes in soil urease activity (μg NH_4^+ released g^{-1} soil hr^{-1})

Treatments	Monsoon fallow period			Cropping period			
	BRF	ARF	EFP	Knee high	Tasseling	Milky	Harvest
T_1	200	275	229	323	266	165	106
T_2	167	306	254	351	279	190	125
T_3	122	262	201	338	238	148	92
T_4	169	298	250	341	276	194	126
T_5	170	301	254	347	276	191	128
T_6	160	280	218	289	262	183	115
T_7	163	267	213	271	255	178	109
T_8	223	327	283	378	341	220	155
T_9	164	305	257	341	277	187	127
T_{10}	69	177	146	196	168	94	67
SEd	3.93	5.46	4.15	7.98	6.54	4.21	2.97
CD (P=0.05)	7.81	10.81	8.21	15.89	12.95	8.34	5.88

BRF: Before rain fall, ARF: After rain fall, EFP: End of fallow period

Table 5. Effect of long term fertilization on dry matter production and yield of maize (kg ha^{-1})

Treatments		Dry matter production			Harvest	
		Knee high	Tasseling	Milky	Straw yield	Grain yield
T_1	50 % NPK	3017	4023	6742	7029	5132
T_2	100 % NPK	3447	4735	7336	8271	5378
T_3	150 % NPK	4101	5719	7526	8514	5492
T_4	100 % NPK + HW	3454	4647	7298	8149	5311
T_5	100 % NPK + Zn	3556	4834	7401	8458	5432
T_6	100 % NP	3492	4663	7311	8134	5213
T_7	100 % N	2651	3526	6036	6933	4256
T_8	100 % NPK + FYM	3896	5475	8015	9379	6057
T_9	100 % NPK (- S)	3378	4591	7316	8221	5349
T_{10}	Control	2240	3055	4567	5242	3012
SEd		61.29	77.35	124.13	165.78	114.32
CD (P=0.05)		121.97	157.32	252.52	328.25	232.52

3.4 Maize Yield and Dry Matter Production

The differential influence of treatments on the dry matter production was very well observed irrespective of the growth stages of maize. In general the application of both increasing levels of inorganic NPK fertilizers along with FYM (T_8) produced significantly higher dry matter production over control emphasizing the beneficial effect of FYM addition. At harvest, the application of optimum NPK fertilizer along with FYM and 100% NPK fertilizer resulted in an increased straw yield to the tune of 13.3 % over 100% NPK. The grain yield of maize crop was highest on application of 100% NPK + FYM (6057 kg ha^{-1}) and the lowest grain yield was recorded in the control plot (3012 kg ha^{-1}). The integrated application of 100% NPK along with FYM at 10 t ha^{-1} recorded the highest yield in all the cropping cycles and showed an increase in grain yield of 12.6 % over 100% NPK. Additions of FYM had directly added an appreciable amount of major nutrient besides micronutrients to the soil which would contribute to the enhanced yield. [38].

4. CONCLUSION

The results derived from the study clearly ascertain that the soil enzymes activity is closely related with the carbon inputs, crop growth stages and abiotic soil environment. The enhanced levels of enzymes activity due to the long term conjoint application of manure and fertilizer promoted the recycling of nutrients and transformation of added nutrients into different labile pools under continuous intensive cropping system and there by maintain the soil fertility in order to sustain soil productivity and biological health over a long run.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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