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Enzyme Activities and Microbial Biomass Carbon in a Soil Amended with Organic and Inorganic Fertilizers

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Abstract: Soil equilibrium can easily be disturbed by unsuitable agricultural practices especially in arid and semi-arid regions which are prone to organic matter losses. A comparative study was conducted to investigate the effects of cow manure, sewage sludge (25 and 100 mg ha^{-1}) and chemical fertilizer (250 kg ha^{-1} of ammonium phosphate and urea) application on Microbial Biomass C (MBC) and enzyme activities in a calcareous soil cropped to corn. Results illustrated that applications of sewage sludge and cow manure increased soil organic C, total N, MBC, L-Glutaminase, alkaline phosphatase, arylsulfatase, β-glucosidase activities and corn yield compared to control and chemical fertilizer treatments. An increasing trend was observed in all studied parameters, as rates of application increased. Manure-amended soils showed higher alkaline phosphatase, arylsulfatase and β-glucosidase activities than that of sewage sludge treatment. Results obtained by discriminant analysis indicated that rates of application were more effective to create discrimination among treatments. It was also understood that β-glucosidase activity was the most useful variable for discriminating among fertilizer types. This study showed that MBC and enzyme activities were significantly correlated with SOC. Significant correlations were also observed between enzyme activities and MBC.

Key words: Microbial biomass, enzyme activities, discriminant functions

INTRODUCTION

Soils from arid and semi-arid regions are not resilient to the effects of inappropriate land use and management which leads to permanent degradation and loss of productivity (Garcia *et al*., 1996). The issue is directly related to the quantity of soil organic matter which is a critical component of soil productivity (Gregorich *et al*., 1996). Soil organic matter formation is a reversible process and most of current agricultural practices are responsible for its reduction in agro-ecosystems, with the consequent decrease of soil biological fertility and soil resilience (Lal, 1994). Soil organic amendments have received renewed attention in recent years due to concerns over adverse effects on agro-ecosystems caused by long-term and continuous use of inorganic fertilizations (Min *et al*., 2003). The use of organic fractions of urban wastes (e.g., sewage sludge and municipal solid wastes) as organic amendments to agricultural soils has been found to be a sound practice to improve soil organic carbon content, water storage capacity and nutrient availability (Chanfigny *et al*., 2000) and an attempt to alleviate the serious environmental and health problems caused by their accumulation.

Recently, it has been established that more dynamic characteristics such as microbial biomass, soil enzyme activities and soil respiration respond more quickly to changes in crop management practices or environmental conditions than do characteristics such as total organic matter (Brookes, 1995). Biological and biochemical attributes may be very dynamic and exceptionally sensitive to changes in soil conditions and that is why there is often a preference for using biological attributes for short term evaluations. Since most of the processes occurring in soils are microbally mediated and are metabolized by enzymes, it is reasonable to suggest that the determination of enzyme activities in soil may be used as a research tool to assess microbial functional diversity, to study biochemical processes, to investigate microbial ecology and ultimately to provide indicators of soil quality (Burns and Dick, 2002). There is also growing interest in using soil enzymes as indicators of soil fertility, since enzymes are sensitive to numerous factors such as climate, type of amendment, crop type and edaphic properties (Naseby and Lynch, 1997). Microbial biomass plays an important role in nutrient cycling and organic matter transformations. It is also sensitive to management effects such as tillage, fertilization and crop rotations (Ndiaye *et al*., 2001).

Using cattle slurry, Paul and Beauchamp (1996) observed a transient in soil microbial biomass soon after a single application to a corn crop whereas Kandeler and

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Eder (1993) reported increases in microbial biomass and enzyme activities involved in N, P and C cycling after long-term addition of cattle slurry to grassland. The objective of this study was to investigate the effects of organic and inorganic fertilizers application on soil biological functionality as described by enzyme activities and MBC.

MATERIALS AND METHODS

A field experiment was started in 1999 on a silty clay loam soil (Typic haplargid: clay, 35.7%; sand, 13.6%; pH 8.3) in Lavark research station (32° 30′ N, 51° 20′ E), Isfahan, Iran. The environmental conditions define the area as arid with an average annual rainfall of 140 mm and a mean annual temperature of 14.5°C. Repeated annual applications of two organic fertilizers (cow manure and sewage sludge) at two levels of application (25 and 100 mg ha^{-1}) were arranged in a complete block design with three replicates. A control and a chemical fertilizer (250 kg ha^{-1} of urea and ammonium phosphate) treatment were also run. Soil and plant sampling were performed in 2003 at the end of the 4th year of application. Composite soil samples were collected from surface soils (0-15 cm) after corn (*Zea mays* L.) harvesting. Soil particle size distribution Soil Organic Carbon (SOC) and Total Nitrogen (TN) using standard procedures were determined (ISRIC, 1986). The relevant characteristics of the studied soil, cow manure and sewage sludge have been shown in Table 1.

Microbial Biomass C (MBC) was determined soon after sampling on field moist soils by the chloroform-fumigation-incubation procedure (Horwath and Paul, 1994).

Table 1: Characteristics of the studied soil, sewage sludge and cow manure

	Unit	Soil	Sewage sludge	Cow manure
pH	-	8.3	6.4	8.6
$^{a}EC_{e\ 25}{}^{°c}$	(dS m^{-1})	1.6	9.4	17
SOC	(g kg^{-1})	5.0	179.8	249.4
TN	(g kg^{-1})	0.77	190.6	130.6
^{b}LG	(mg N-NH_4^+ kg^{-1} soil 2 h)	185.5	146.3	153.6
^{c}AK	(mg PNP kg^{-1} soil h)	117.1	111.6	121.4
^{d}AS	(mg PNP kg^{-1} soil h)	238.6	118.6	132.7
^{e}βG	(mg PNP kg^{-1} soil h)	59.7	83.6	155.9

^{a} $EC_{e\ 25}{}^{°c}$ (electrical conductivity), ^{b} LG (L-Glutaminase), ^{c} AK (alkaline phosphatase), ^{d} AS (arylsulfatase), ^{e} βG (β-glucosidase)

Hydrolases, L-Glutaminase (LG), Alkaline Phosphatase (AP), arylsulfatas (AS) and β-glucosidase (βG) activities were determined according to standard procedures described by Tabatabai (Tabatabai, 1994), using L-glutamine (50 mM, pH 10) ρ-nitrophenyl phosphate (10 mM, pH 11), ρ-nitrophenyl sulfate (10 mM, pH 5.8) and ρ-nitrophenyl β-D-glucopyranoside (10 mM, pH 6) as the substrates, respectively. All measurements were expressed on a moisture free basis.

Statistical analysis: Analysis of variance (ANOVA) and Least Significant Difference (LSD) was determined by SAS 8.2 software. Discriminant Analysis (DA), linear regression and Pearson correlation coefficients were calculated using SYSTAT 8.0 software.

RESULTS

SOC and TN: Results illustrated that SOC and TN were greater in soils amended with sewage sludge and cow manure as compared to chemical fertilizer and control treatments (Table 2). It was observed that sewage sludge application increased TN to higher levels than cow manure (Table 2). Cow manure and sewage sludge treated soils showed to be similar in terms of SOC (Table 2).

Soil microbial biomass: Organic fertilizer application increased MBC compared to chemical fertilizer and control treatments, but there was no significant difference between manure-and sludge-treated soils (Table 2). Increasing levels of application increased MBC in both manure-and sludge-amended soils (Table 3). The lowest amount of MBC was observed in control treatment (Table 2). Soil organic C (r = 0.84**), TN (r = 0.86**), LG (r = 0.947**), AP (r = 0.92**), AS (r = 0.84**) and βG (r = 0.73**) activities were significantly correlated with MBC. There was also significant correlation between MBC (r = 0.78**) and corn yield (Table 4).

L-glutaminase (LG) activity: Among hydrolases, LG plays an important role in supplying N to plants (Tabatabi, 1994). Results showed that the activity of this enzyme was significantly higher in soils received sewage sludge and cow manure than that of chemical fertilizer treatment. However, there was no significant difference

Table 2: Soil Organic Carbon (SOC), Total Nitrogen (TN), Microbial Biomass C (MBC), L-Glutaminase (LG), Alkaline Phosphatase (AP), arylsulfatase (AS) and β-glucosidase (βG) activities and corn yield as affected by organic and chemical fertilizers

Type of amendment	SOC (g kg^{-1})	TN (g kg^{-1})	MBC (mg kg^{-1})	LG (mg N-NH_4^+ kg^{-1} soil 2 h^{-1})	AK (mg PNP kg^{-1} soil h^{-1})	AS (mg PNP kg^{-1} soil h^{-1})	βG (mg PNP kg^{-1} soil h^{-1})	Yield ($Mgha^{-1}$)
CM	22.7a	2.8b	1383.1a	316.4a	412.3a	557.0a	455.5a	60.3a
SS	22.1a	3.4a	1348.3a	312.4a	375.3b	497.4b	182.7b	57.5a
CF	5.1 b	1.0c	722.0b	202.7b	138.1c	457.6c	92.0c	38.7b
CT	4.9 b	0.8c	606.7c	185.5b	117.1d	238.6d	59.7d	19.8b

(CM = Cow Manure, SS = Sewage Sludge, CF = Chemical Fertilizer and CT = Control); * Different letters in each column indicate significant difference between treatments according to Duncan test ($p<0.05$)

Table 3: Soil parameters as affected by application rates

	(0 mg ha^{-1})	(25 mg ha^{-1})	(100 mg ha^{-1})
SOC (g kg^{-1})	4.9b	18.6b	43.9a
TN (g kg^{-1})	0.8c	2.6b	6.2a
MBC (mg kg^{-1})	606.7c	1533.2b	2009.4a
LG (mg N-NH_4^+ kg^{-1} soil 2 h)	185.5b	324.5b	441.8a
AK (mg PNP kg^{-1} soil h)	117.1c	322.8b	751.9a
AS (mg PNP kg^{-1} soil h)	238.6c	531.1b	921.5a
βG (mg PNP kg^{-1} soil h)	59.7c	215.6b	698.2a
Yield (Mg ha^{-1})	19.8b	56.1b	110.1a

* Different letters in each row indicate significant difference between treatments according to Duncan test ($p<0.05$)

Table 4: Correlation matrix for pooled data

Parameter	1	2	3	4	5	6	7	8
SOC	1							
TN	0.96**	1						
MBC	0.92**	0.86**	1					
LG	0.92**	0.85**	0.94**	1				
AK	0.98**	0.94**	0.92**	0.91**	1			
AS	0.92**	0.87**	0.84**	0.87**	0.96**	1		
βG	0.74**	0.62**	0.73**	0.80**	0.82**	0.90**	1	
Yield	0.91**	0.84**	0.78**	0.81**	0.88**	0.83**	0.71**	1

** Significant at ($p<0.01$)

Table 5: Discriminant analysis for TN, SOC, Soil enzyme activities, MBC and corn yield measured in the 0-15 cm depth of the studied plots

	Types of amendments		Rates of application	
	Factor 1	Factor 2	Factor 1	Factor 2
Eigenvalue	13.86	2.4	612.31	22.54
Dispersion (%)[a]	85.3	100	96.5	100
Lambda[b]	0.02, $p<0.001$		0.000, $p<0.001$	
Standardized coefficients[c]				
SOC	0.354	1.661	1.418	-0.178
TN	0.286	0.223	0.94	0.277
MBC	ND[d]	ND	0.623	-2.194
LG	3.59	-0.894	-1.567	0.584
AK	-3.694	-5.109	2.252	1.144
AS	5.672	4.54	-1.206	0.715
βG	-6.139	-1.029	ND	ND
Yield	ND	ND	-0.708	0.057

[a]Cumulative dispersion as proportion of total dispersion, [b] Wilks lambda = multivariate test of dispersion among all the groups on all the variables, [c]Coefficients of discriminant functions were standardized by within variances using the transformed squared root of SOC, TN and enzyme activities except for β-glucosidase, [d]ND = not determined as variables showed to be redundant and were not used in the model

between sludge-and manure-treated soils (Table 2). It was observed that LG activity increased as rates of application increased (Table 3). The lowest level of LG activity was observed in control treatment (Table 2).

Alkaline Phosphatase (AP) activity: Organic phosphorus in soil can comprise 30 to 70% of the total phosphorus content. Hydrolysis of these organic phosphorus compounds is essential for uptake by plants and microorganisms, which is carried out by AP activity especially in calcareous soils (Tabatabai, 1994). The application of both sewage sludge and cow manure enhanced AP activity when compared with chemical fertilizer and untreated soils. In contrast to LG, AP activity was significantly higher in manure-treated soils than that of sewage sludge treatment. The lowest AP activity was observed in control treatment (Table 2). Similar to LG activity, an increasing trend was observed as rates of application increased.

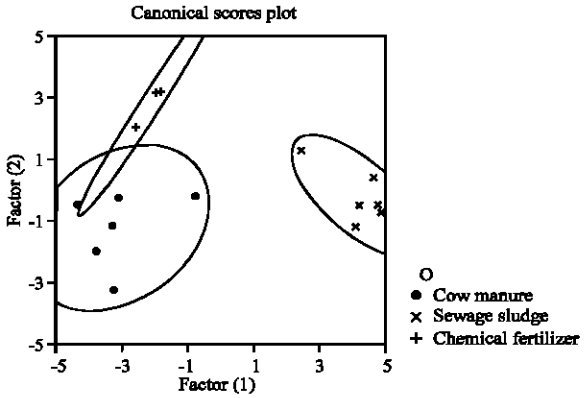

Fig. 1: Two-dimensional plots of the discriminant analysis for the activities of soil nutrient mineralizing enzymes in the 0-15 cm depth and grouped by type of fertilizers

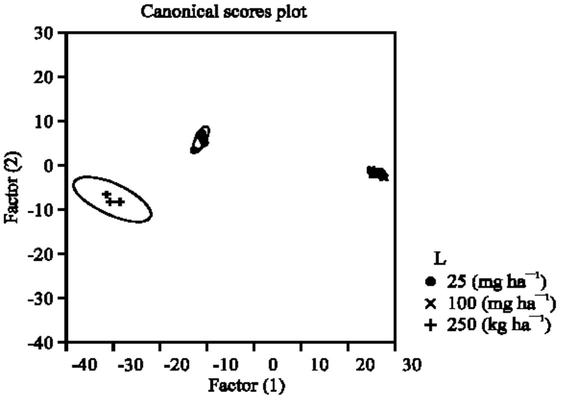

Fig. 2: Two-dimensional plots of the discriminant analysis for the activities of soil nutrient mineralizing enzymes in the 0-15 cm depth and grouped by level of application

Arylsulfatase (AS) activity: A large fraction (40-70%) of the total sulfur content in soil may be present as ester sulfate. Arylsulfatases play an important role in the mineralization of sulfur (Tabatabai, 1994). The activity of this enzyme followed a pattern similar to AP activity (Table 2 and 3).

β-glucosidase (βG) activity: The hydrolysis products of β-glucosidases are believed to be important energy sources for soil microorganisms (Tabatabai, 1994). Similar to AP and AS activities, βG activity in cow manure applied soils was significantly higher than that of sewage sludge treated soils. Interestingly, βG activity showed a more pronounced difference between sludge-and manure-amended soils (Table 2). A similar pattern was observed for βG activity as level of application increased (Table 3). The highest value of βG activity was distinguished in 100 mg ha^{-1} followed by 25 mg ha^{-1} (organic fertilizer application) and 250 kg ha^{-1} (chemical fertilizer application). Alkaline Phosphatase (AP), LG, AS and βG activities were positively correlated with SOC, TN and corn yield (Table 4).

Discrimination among treatments according to their properties: Discriminant Analysis (DA) was used to determine the most useful variables for discriminating among groups and to test for differences or similarities between groups. Discriminant analysis indicated that the studied parameters could be separated according to the type of fertilizers and application rates (Table 5 and Fig. 1, 2). Results also represented that between rates of application and type of fertilizers, rates of application was more effective to create discrimination among treatments (Fig. 1 and 2).

DISCUSSION

SOC and TN: Soil organic matter plays an important role in maintaining soil fertility. The decrease in soil organic matter is paralleled by declines in soil fertility (Tate, 1987). An enhance in SOC and TN content under organic fertilizer application is possibly due to high loadings of organic C and N in these organic materials (Table 1), efficient metabolic activity of microorganisms and physico-chemical protection of organic C and N in soil (Oades, 1984).

Microbial biomass: Determination of soil MBC content is generally used as a rapid indicator of the response of microbial biomass to changes in soil management that affect the turnover of organic matter (Nannipieri *et al*., 1990). Results of this study implied that soils amended with sewage sludge and cow manure increased MBC significantly more than what observed for chemical fertilizer and control treatments (Table 2). A relatively rapid response to organic amendments has been reported for MBC by several workers, which suggests it could be useful indicator in identifying positive effects of soil management (Fauci and Dick, 1994a). Addition of organic fertilizers by preparing microbial habitats, increasing more food resources and directly addition of soil organisms via incorporation of cow manure and sewage sludge might enhance microbial biomass in this experiment. Other authors have reported a similar effect on soil microbial biomass (Garcia *et al*., 1998). Since the availability of organic C is considered the limiting factor for the size of MBC (Min *et al*., 2003), a reduction in MBC was presumably due to lower availability of C in long-term application of inorganic fertilizers into the soils. Others have reported that soils receiving manures have larger MBC pool than in the same soils receiving only chemical fertilizers (Islam and Weil, 2000). There was more MBC in soils under higher rate of organic fertilizer application (100 mg ha^{-1}) as compared to soils under chemical fertilizer and control treatments (Table 3). Increasing MBC after application of these amendments at a higher rate of application suggests that this soil is C-limited and that the labile C provided by cow manure and sewage sludge may have been used as an energy source for microorganisms. As MBC is controlled by availability of C content, greater amounts of organic matter in both sewage sludge and cow manure could have provided more labile C substrates needed for maintenance of the larger MBC in sewage sludge and cow manure applied soils (Min *et al*., 2003).

Enzyme activities: Soil is dynamic living system where all biochemical processes are proceeding through enzymatic activities (Tabatabi, 1994). Results of this study showed that application of cow manure and sewage sludge increased enzyme activities when compared with chemical fertilizer and untreated soils (Table 2). The activities of all studied enzymes as compared to control and chemical fertilizer treatments reached a maximum by increasing rates of organic fertilizer incorporation from 25 mg ha^{-1} to 100 mg ha^{-1} (Table 3). This implies that edaphic conditions created by 100 mg ha^{-1} application rates were more favorable to the growth of microorganisms and plant roots that secrete enzymes into the soil. The level of soil enzyme activity increases with increasing soil organic matter content (Speir, 1977). Although, different types of soil amendments can contain enzymes (Table 1), the stimulation of enzyme activity is likely to be related to increased microbial growth (Martens *et al*., 1992), because introduced enzymes are rapidly degraded and denatured in soils (Dick *et al*., 1983). Repeated inorganic N fertilizer application in the greenhouse over a period of 305 days showed no significant effects on β-glucosidase and protease activities in soil whereas organic amendments stimulated enzyme activities (Fauci and Dick, 1994b). Regardless of LG activity, addition of cow manure showed higher AP, AS and βG activities than that of sewage sludge treatment. Interestingly, increasing in βG activity under application of cow manure was three times more than sewage sludge application (Table 2). The increased level of βG activity in manure-amended soils to a higher degree than sludge-treated soils can be related to the fact that biochemical compositions of these materials might be different. Since cow manure probably posses higher contents of cellulose compounds, application of cow manure may induce microbial βG production in manure-applied soils. Enhancing enzyme activities after long-term application of farmyard manures have been announced by several workers (Kandeler and Eder, 1993). These studies have often shown that manure-amended soils have higher microbial biomass, N-mineralization potential and microbial activities that are related to some enzyme activities. This would be expected as elevated levels of C inputs and other nutrients would stimulate biological activity and stabilization of abiontic enzymes.

The activity of soil enzymes could be inhibited by using a certain kind of organic amendments. Bonmati *et al*. (1985) have reported that the presence of heavy metals in sewage sludge were responsible for the inhibitory effects when amended soil was assayed for urease and phosphatase activity.

The close relationship between enzyme activities with SOC and TN (Table 4) may be related to both the adsorption of extracellular enzymes on humic substances or to the promoting effects of SOC and TN on soil microbial biomass and intracellular enzymes (Baligar *et al*., 1991). Strong correlations between MBC and LG (r = 0.94**), AP (r = 0.92**), AS (r = 0.84**) and βG (r = 0.73**) activities confirmed the idea that microbial activities are the main source of enzyme production to the soils (Tabatabi, 1994). There were also significant relationships between studied enzyme activities. The significant correlation between these enzymes is not unusual because similar relationships have been reported for other soil enzymes. For instance, Speir (1977) found that soil AS activity significantly correlated with phosphatases and urease activities. Significant correlations were also observed between MBC (r = 0.78**), LG (r = 0.81**), AP (r = 0.88**), AS (r = 83**) and βG (r = 0.71**) activities with corn yield. Relating soil enzyme activities to plant productivity has produced mixed results (Dick, 1996). Strong correlation between enzyme activities and plant productivity in this study adapted to the hypothesis that application of organic fertilizers by supplying essential nutrients for plant uptake through enzymatic processes can increase crop yields. It is also suggested that improving soil structure following addition of organic materials could be an effective factor in increasing plant productivity.

Discrimination among treatments according to their properties: Multivariate DA was used to determine the relevance of SOC, TN, MBC and the four soil enzymes for explaining the impact of application rates and fertilizer types on the functionality of microbial communities. Preliminary DA showed us that MBC and corn yield were redundant variables for discriminating among fertilizer groups and βG activity for discriminating among application rates and therefore eliminated from the final multivariate discriminant function model. The Jacknifed classification matrix obtained from DA for the influence of fertilizer types represented that the discriminant functions were able to correctly predict 83% of the variances among parameters grouped in the cow manure, 100% in sewage sludge and 67% of the total dispersion in chemical fertilizer group (data not shown). The eigenvalues obtained from DA for the influence of type of fertilizers showed that the first canonical factor (factor 1) captured most of the differences among the three different type of fertilizers and expressed 85.3% of the total variances of the data set (Table 5). The contribution of the factor 2 was minor and captured 14.7% of the dispersion. Based on the standardized coefficients of the discriminant functions, βG, AS, AP and to a lesser extent LG activities were the most important variables driving factor 1 (Table 5). In this experiment, the contribution of βG activity illustrated that the supply of soil organic substrates such as cellobiose increases from chemical fertilizer to sewage sludge and cow manure application. These finding supports the idea that the supplies of substrates containing C stimulate nutrient cycling in agro-ecosystems (Monreal and Bergstrom, 2000) especially in those located in arid and semiarid regions which are prone to scarcity of organic matter content. Findings of this study also support the concept that measurements of soil hydrolases provide an early indication of changes in soil fertility, since they are related to the mineralization of essential nutrient elements such as N, P, C and S (Ceccanti and Garcia, 1994).

Analysis of the SOC, TN, MBC, yield and the three enzymes measured implied a high discrimination among 25 mg ha^{-1}, 100 mg ha^{-1} and 250 kg ha^{-1} (Fig. 2). By comparing results obtained from DA for fertilizer types and application rates, it can be realized that the studied variables are more sensitive to the application rates than to fertilizer types. The Jacknifed classification matrix illustrated that the soil samples collected from plots under different rates of application, were able to correctly anticipate 100% of the variances among parameters grouped in three different rates of application. The eigenvalues representing the influence of application rates indicated that the first canonical variable (Factor 1) captured 96.5% of the total data dispersion. Factor 1 discriminated better between 25 mg ha^{-1} versus. 100 mg ha^{-1} and 250 kg ha^{-1} groups. Factor 2 only contributed to 3.5% of total data dispersion. The most important parameters driving Factor 1 as shown by their standardized coefficients were AP followed by LG activity and SOC. Contribution of SOC to the final model suggests that this soil is C-limited and that the studied soils can be separated by their organic carbon content when they are treated with different rates of application. The most striking feature of the data set was that the response of soil microbial communities to the amendments changed due to ecological succession processes from inorganic to organic fertilization. It was interestingly observed that soils received higher amount of organic materials showed more microbial functions. Therefore, the soil microbiological characteristics (MBC and enzyme activities involved in C, N, P, S cycling) selected for doing present study provided a reliable research tool to estimate early changes in the dynamics of soil microbial processes after improving soil organic matter content. Studies from long-term sites have shown that soil enzyme activities are sensitive in discriminating among soils which have been treated with different organic amendments such as animal manure (Martens *et al*., 1992) and municipal refuses (Perucci, 1992).

To conclude, it seems that addition of both organic fertilizers (cow manure and sewage sludge) increased SOC, TN, MBC and the key enzyme activities that would contribute to the various soil functions and that the different enzymes have not responded similarly to the fertilizer treatments. The enhanced levels of soil enzyme activity and MBC upon application of cow manure and sewage sludge to the soils promoted the recycling of nutrients and energy in the soil ecosystem. It was also observed that land application of sewage sludge can return valuable nutrients and organic matter to the land, but its usage should be controlled to cut the risks of heavy metal accumulation in applied soils. By DA performance it was observed that βG, AP and AS activities explained the influence of fertilizer types and SOC, AP and LG activities expressed the influence of application rates on the ability of soils to supply essential nutrient elements for microbial communities present in soil. β-glucosidase activity best expressed the influence of fertilizer types and alkaline phosphatase activity the influence of application rates.

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