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Effect of long term application of organic and inorganic fertilizers on soil microbial activities in semi-arid and sub-humid rainfed agricultural systems

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Abstract: A study was conducted to investigate the effect of chemical fertilizer, organic manure and two cropping systems viz., pearlmillet - clusterbean - castor rotation and upland rice - lentil sequence on microbial quotient (MQ), metabolic quotient, specific enzyme activity (dehydrogenase, arylsulfatase and urease) and microbial biomass carbon (MBC) in long-term (18-21 years) field experiments in Entisols of semi-arid region of Gujarat and Inceptisols of sub-humid region of Varanasi. Higher MQ values were recorded in Entisols than Inceptisols. MQ ranged from 4.00–5.08 and 1.00–1.85 % across soil layers in Entisols and Inceptisols, respectively. Metabolic quotient values ranged from 0.11–0.23 and 0.04–0.07 across soil layers in Entisols and Inceptisols respectively. The specific enzyme activity of dehydrogenase was maximum in Entisols, whereas, arylsulfatase and urease activity was recorded more in Inceptisols. Higher specific enzyme activity reflects greater microbial activity and microbial biomass turnover. Agro-ecosystem in sub-humid region resulted in 1.5 fold higher geometric mean of enzymes (GMea) than in semi-arid region. The application of 50% RDNF (recommended dose of N-fertilizer) + 50% RDN FYM (farm yard manure) in Entisols and 50% N (FYM) + 50% RDF in Inceptisols improved microbiological activities (GMea) at both the sites. The concentration of soil organic carbon (SOC) and MBC were significantly correlated with GMea in both agro-ecosystems. In conclusion, integrated sources of nutrients with inclusion of FYM and 50 % reduction of fertilizers improved the microbiological activities in both Inceptisols and Entisols.

Key words: Drylands, entisol, inceptisol, long-term fertilization, soil enzymes.

Introduction

Application of chemical fertilizers to soil is the most common practice to increase crop yields. Indiscriminate use of fertilizers has led to changes in soil physical, chemical and biological characteristics (Rivera-Becerril *et al.* 2017). The problems they are causing on soil and water has become one of the

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Table 1. Climatic and edaphic characteristics of two experimental sites.

Parameter	Site 1 (Dryland Research Station, SK Nagar, Gujarat)	Site 2 (Varanasi, Uttar Pradesh)
Location	24°30´N, 72°13´E, 152.5 MASL	25°11´N, 82°51´E, 480 MASL
Duration of experiment (years)	18 (1988–2005)	21 (1986–2007)
Climate	Semi-arid	Sub-humid
Cropping system	Pearlmillet-clusterbean-castor rotation (each crop once in 3 years)	Rice (upland)-lentil sequence
Soil order and type	Entisol	Inceptisol
Annual rainfall (mm)	550	1080
Annual minimum temperature (° C)	18.7	26.6
Annual maximum temperature (° C)	30.2	34.4
Length of growing period (days)	60–90	150–180
Bulk density (g cc^{-1}), soil texture (sand, silt & clay %), Soil organic carbon (SOC-g kg^{-1}) soil pH, available N, P, K (kg ha^{-1})	1.42, 85.4, 4.0, 10.6, 2.0, 8.2, 114.2, 18.4, 94.1	1.49, 58.2, 14.0, 27.8, 1.4, 6.7, 160, 21.2 & 119
Treatments		
T1	Control (no fertilizer, no amendments)	Control (no fertilizer, no amendments)
T2	100 % RDNF (recommended dose of N fertilizer, i.e., 80, 20 and 60 kg N ha^{-1} was applied as urea to pearl millet, cluster bean and castor respectively)	100% RDF (recommended dose of NPK through mineral fertilizer 60:50:30 kg N:P_2O_5:K_2O ha^{-1})
T3	50% RDN(F) (through fertilizer)	50% RDF
T4	50% RDN(FYM) (recommended dose of N through farmyard manure)	100% organic (FYM) (N equivalent)
T5	50% RDN(F) + 50% RDN(FYM)	50% RDF + 50% RDF (foliar)
T6	Farmers method (5 Mg of FYM ha^{-1} once in 3 years)	50% organic (FYM) (N equivalent) + 50% RDF
T7	-	Farmers' practice (20 kg N ha^{-1} only)
Replications	3	3
Experimental Design	Randomized block design (RBD)	Randomized block design (RBD)

biggest environmental concerns (Singh *et al.* 2017). Soil microorganisms are key players in terrestrial agroecosystems, involved in soil organic matter decomposition, nutrient cycling, bioremediation of pollutants and sustaining soil productivity (Dong *et al.* 2014; Luo *et al.* 2015). Agricultural practices have significant influence on the soil microbial activities and health of soils. Continuous application of fertilizers has changed the composition and functions of soil microorganisms (Dong *et al.* 2014). Activity of soil enzymes is widely used as an indicator of soil quality (Salazar *et al.* 2011). They play crucial role in soil organic matter (SOM) decomposition and nutrient cycling (Nannipieri & Paul 2009). Soil microorganisms and plant roots are the major source of soil enzymes. Dehydrogenase activity is a generalized indicator of soil catabolic activity reflective of carbon cycling and arylsulfatase and urease are involved in sulfur (S) and nitrogen (N) cycles respectively (Das & Varma 2011). Limiting the use of chemical fertilizers is imperative for the sustainability of cropping systems (Drinkwater & Snapp 2007). Long term studies are essential to understand the effect of different management practices on soil and developing location specific sustainable crop production systems. They are probably the only means to directly quantify the influences of diverse management practices on soil properties. Tropical drylands, particularly in India, are characterized by high evapotranspiration (ET), low soil organic carbon (SOC), high soil temperatures and relatively infertile and erosion-prone soils (Srinivasarao *et al.* 2014). Inceptisols of Indo-Gangetic plains are very deep, loamy to sandy or silty in texture (Bhattacharyya *et al.* 2013). Entisols of Gujarat are formed from basaltic, gneissic, granitic and alluvial parent material. They are light grey to dark grey, reddish brown to yellowish brown in colour (Merry & Chamyal 1997). Very little information is available on the influence of long term fertilization and cropping pattern on soil microbial indices of soil quality e.g., microbial biomass carbon, dehydrogenase, soil enzyme activity in rain fed ecosystems and how they relate to crop and nutrient management practices. Therefore, the present investigation was undertaken with the principal objective of evaluating the effects of long-term nutrient management practices on soil microbial activities in two diverse cropping systems in two major soil types in semi-arid and sub-humid tropical India.

Material and methods

Details of experimental sites and treatments

Two field experiments were conducted in semi-arid north Gujarat plain (Sardar Krushi Nagar, Gujarat) and sub-humid Rohilkhand, Awadh and south Bihar plain (Varanasi, Uttar Pradesh) regions of India representing Entisol and Inceptisol soils having pearlmillet-clusterbean-castor rotation and upland rice-lentil sequence, respectively. Information regarding locations, climate, soil properties and treatment details are furnished in Table 1. Common soil fertility management treatments across two experiments were control (no fertilizer or organics), 100% recommended dose of fertilizers (RDFs), 50% RDF+50% organics, and 100% organics. At Sardar Krushi Nagar, Gujarat, a shallow ploughing (0.15–0.20 m deep) followed by blade harrowing was done every year after the first rainfall during the last week of June before planting of the crop. At Varanasi, upland rice was grown during the rainy season (June-September) followed by lentil in the post-rainy (October-December) season on residual fertility.

Soil sampling

Soil samples were taken from 3 depths (0–0.20 m, 0.20-0.40 m and 0.40–0.60 m) during May 2006 and April 2007 in SK Nagar and Varanasi, respectively. Three random samples were taken in each plot using a tube auger and composited depth-wise. The samples were hand-crushed, passed through a 2-mm sieve and stored at 4 ^{o}C until analysis.

Soil physical and chemical analysis

Bulk density, soil texture, Soil organic carbon (SOC) soil pH, available N, P & K were analysed (Page *et al.* 1982). All determinations were performed in triplicate, and the results expressed on oven-dry weight basis.

Analysis of microbial parameters

Soil microbial biomass carbon (MBC) was determined by the fumigation–extraction method (Vance *et al.* 1987). Dehydrogenase (DHA) was spectrophotometrically measured using 2, 3, 5-triphenyl tetrazolium chloride (TTC) as electron acceptor (Casida *et al.* 1964), Arylsulfatase (AS) activity was measured with p-nitro phenyl sulphate as substrate (Tabatabai & Bremner 1970), urease activity was assayed by incubating the soil with urea (Tabatabai & Bremner 1972). All determinations were performed in triplicate and results were expressed on oven dry basis. The profile mean enzyme activity was calculated by averaging the values for all three soil depths (0–0.2, 0.2–0.4, 0.4–0.6 m).

Microbial quotient and metabolic quotient

Microbial quotient was calculated as a ratio of microbial biomass carbon and soil organic carbon. Since soil respiration was not directly measured, dehydrogenase activity was taken as a surrogate and metabolic quotient was thus calculated as a ratio of dehydrogenase activity over microbial biomass carbon.

Specific enzyme activity

Specific soil enzyme activity of soils was calculated by dividing enzyme activities over soil organic carbon content (Trasar-Cepeda *et al.* 2008).

Geometric mean of enzymatic activities (GMea)

The GMea is a soil quality index which integrates the information from all enzymes and their specific range of variation (Paz-Ferreiro *et al.* 2012). This general index was calculated for each treatment and soil depth (0–0.2, 0.2–0.4 and 0.4–0.6 m) using Equation:

$$GMea = (DHA \times ASA \times UA)^{1/3}$$

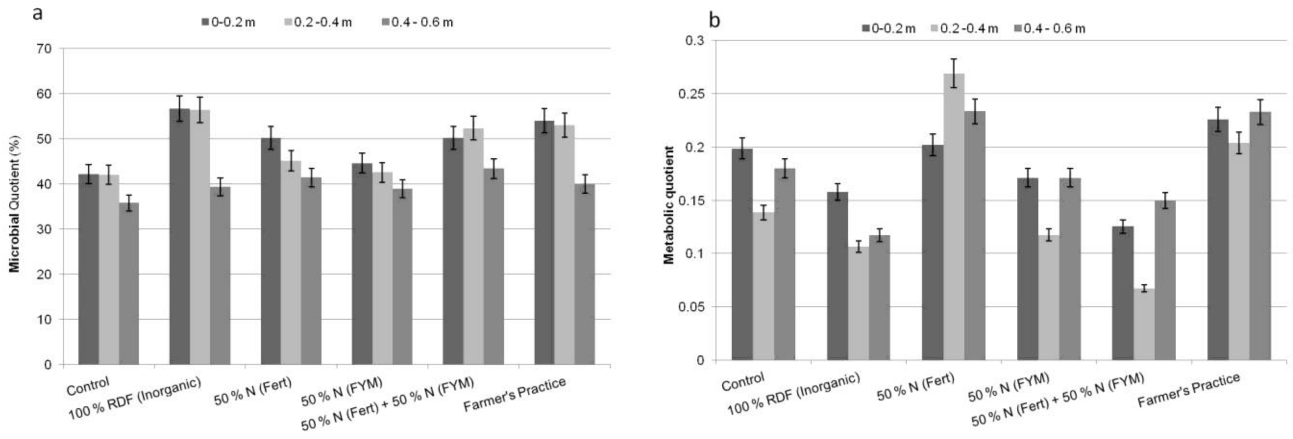

Fig. 1. Effects of long term nutrient management practices on (a) Microbial quotient-MBC/SOC (b) Metabolic quotient (DHA/MBC) in semi arid region Entisols of S.K. Nagar. MBC-Microbial biomass carbon, SOC-Soil organic carbon, DHA-dehydrogenase.

Statistical analyses

Statistical analyses of the data were done using Statistical Packages for Social Sciences (2001) to find out variance and to determine the statistical significance of the treatment effects. Duncan's multiple range test (DMRT) was used to compare the treatment means. Simple correlation coefficients and regression equations were also computed (SPSS 2001), to evaluate the relationships among the response variables and the enzyme activity at 95% probability level.

Results

Effect of long-term cropping and nutrient management on soil microbial parameters

Microbial quotient and Metabolic quotient (DHA/MBC)

Recommended dose of nitrogen fertilizer and farmers' practice (5 Mg FYM ha^{-1} once in 3 years) recorded the highest microbial quotient across soil depths analyzed under Entisols with pearl millet-cluster bean-castor rotation. Lowest value was observed in T1 (Control - no fertilizer, no amendments) (Fig. 1a). Under Inceptisols with upland rice-lentil sequence, maximum microbial quotient value was observed in T6 (50% organic (FYM) - N equivalent) + 50% RDF) and T2 (100% RDF). Lowest value was recorded in T1 (Fig. 2a).

Organic fertilizer (FYM) and reduced dose of chemical fertilizer resulted in improving the metabolic quotient under Entisols with pearl millet-cluster bean-castor rotation as compared to the use of sole mineral fertilization. Maximum value (i.e, mean of 3 layers) was recorded in 50% RDNF treatment and lowest value was observed in control (no fertilizer and no amendments) (Fig. 1b). Under Inceptisols with upland rice-lentil sequence, highest value was recorded in T7 (Farmers' practice (20 kg N ha^{-1} only) and lowest value was observed in T5 (50 % RDF + 50 % RDF – foliar) (Fig. 2b).

Specific enzyme activities

Dehydrogenase (DHA/SOC), Arylsulfatase (AS/SOC) and Urease (Urease/SOC)

Sole organic (FYM) source of nutrients and 50% reduction in chemical nitrogenous fertilizer recorded maximum specific enzyme activity i.e., dehydrogenase in Entisols. Highest value was recorded in farmers practice (5 Mg of FYM ha^{-1} once in 3 years) and lowest value was observed in T5 (50% RDNF + 50% RDN-FYM) (Fig. 3a). Under Inceptisols, recommended dose of chemical fertilizer recorded highest specific dehydrogenase activity and lowest value was observed in 50% RDNF (Fig. 4a).

Under Entisols with pearl millet-cluster bean-castor rotation, highest specific enzyme activity i.e., arylsulfatase was recorded in farmers practice (5 Mg of FYM ha^{-1} once in 3 years) and lowest value was observed in control (Fig. 3b). In Inceptisols with rice-lentil cropping sequence, highest value was recorded in farmers' practice (20 kg N ha^{-1} only) and lowest value was observed in T5 (50% RDF + 50% RDF - foliar) (Fig. 4b).

Reduced dose of mineral fertilizer recorded maximum specific enzyme activity i.e., urease under

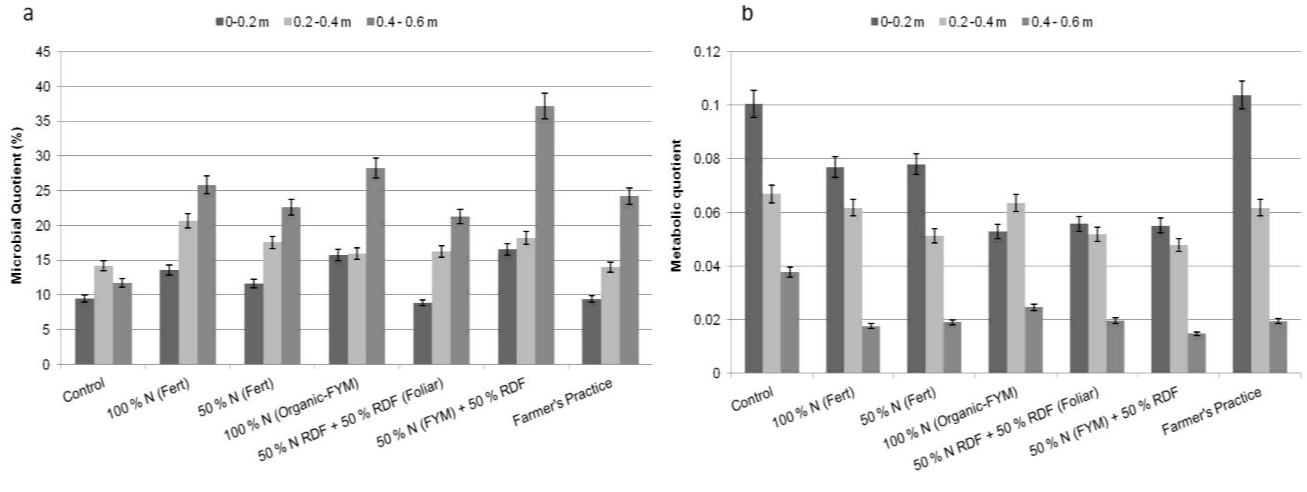

Fig. 2. Effects of long term nutrient management practices on (a) Microbial quotient-MBC/SOC (b) Metabolic quotient (DHA/MBC) in Inceptisols of sub humid region of Varanasi. MBC-Microbial biomass carbon, SOC-Soil organic carbon, DHA-dehydrogenase.

Entisols with pearl millet-cluster bean-castor rotation. Highest value was recorded in 50% RDNF treatment and lowest value was observed in 100% RDNF treatment (Fig. 3c). Under Inceptisols with rice-lentil cropping sequence, highest value was recorded in 100% RDNF treatment and lowest value was observed in 100 % FYM treatment (Fig. 4c).

Geometric mean

Under Entisols with pearlmillet-cluster bean-castor rotation, the treatment with 50% RDNF + 50% RDN-FYM recorded highest value and control (no fertilizers, no amendments) recorded lowest value (Table 2). In Inceptisol with rice-lentil cropping system, 50% organic-FYMN equivalent + 50% RDF recorded highest value and control recorded lowest value. The highest GMea was observed with integration both organic and inorganic nutrient sources at both the sites (Table 3).

Response of soil enzymes to SOC and MBC

Concentrations of SOC and MBC significantly influenced the GMea at both sites (Fig. 5). A positive correlation was observed between absolute enzyme activity, soil organic carbon and microbial biomass carbon in both semiarid region Entisol and sub humid region Inceptisol (Table 4). A reduction in the proportion of MBC to OC due to certain nutrient management practice indicates decrease in microbial biomass in microbial transformation processes (Rao 2013).

Discussion

Soil quality pertains to physical, chemical and biological characteristics of soil and they facilitate diverse functions (Cheg *et al.* 2014; Fang *et al.* 2014). It is the biological parameters that react most rapidly to altered soil conditions than the physical and chemical characteristics (Paz-Ferreiro *et al.* 2012). Soil enzymes are primarily produced by microorganisms and are key drivers of organic matter decomposition and nutrient cycling (Burns *et al.* 2013). They are good indicators of soil productivity as they clearly display the effects of fertilization and other crop management practices (Paz-Ferreiro *et al.* 2012).

Regional variations involving climate, soil type and hydrothermal conditions of two experimental locations at Gujarat and Varanasi significantly impacted the soil enzymatic activity. MQ denotes the role of microbial biomass in improving the SOC content (Sparling 1992; Yang *et al.* 2010). It is used as an indicator of organic matter with respect to changes in soil properties (Sparling 1992). In the present study, the ratio values ranged from 4.00–5.08 and 1.00–1.85% across soil layers in Entisols and Inceptisols respectively. Lower MQ values in Inceptisols was due to less availability of nutrients for microbial growth, and also reduced conversion of organic carbon to microbial biomass (Novak *et al.* 2017). Higher MBC in Entisols over Inceptisols has resulted in higher MQ values in the present investigation. Soil particles such as clay influences

Table 2. Geometric mean of the enzymes as influenced by nutrient management practices in Entisols under semi-arid conditions.

Treatments	Geometric mean			
	0.0–0.2 m	0.2–0.4 m	0.4–0.6 m	Profile Mean
T1	4.9±0.3^{Da}	3.5±0.2^{Eb}	2.9±0.1^{Ec}	3.8±0.2^{D}
T2	7.2±0.4^{Ba}	5.7±0.3^{Bb}	4.4±0.3^{Cc}	5.7±0.3^{B}
T3	6.4±0.3^{Ca}	5.4±0.3^{Cb}	4.0±0.2^{Dc}	5.3±0.3^{C}
T4	7.4±0.4^{Ba}	4.3±0.2^{Dc}	5.1±0.3^{Ab}	5.6±0.3^{B}
T5	8.6±0.4^{Aa}	8.0±0.4^{Ab}	4.9±0.3^{ABc}	7.2±0.4^{A}
T6	6.5±0.4^{Ca}	5.9±0.3^{Bb}	4.8±0.2^{Bc}	5.7±0.3^{B}
Mean	6.8	5.5	4.4	5.5

Different capital letters within columns and small letters within rows are significantly different at P=0.05 according to Duncan Multiple Range Test (DMRT) for separation of means.

Table 3. Geometric mean of the enzymes as influenced by nutrient management practices in Inceptisols under sub-humid conditions.

	Geometric mean			
	0.0–0.2 m	0.2–0.4 m	0.4–0.6 m	Profile Mean
T1	7.2±1.1^{Da}	6.1±0.8^{Cb}	4.2±0.4^{Cc}	5.8±0.8^{D}
T2	13.3±2.6^{Ba}	9.0±1.4^{Ab}	5.4±0.6^{Bc}	9.2±1.5^{B}
T3	10.2±1.8^{Ca}	7.3±1.0^{Bb}	5.1±0.5^{Bc}	7.5±1.1^{C}
T4	12.5±2.4^{Ba}	8.5±1.3^{Ab}	7.0±1.0^{Ab}	9.4±1.6^{B}
T5	9.8±1.6^{Ca}	7.1±1.0^{Bb}	5.6±0.7^{Bc}	7.5±1.1^{C}
T6	16.5±3.1^{Aa}	9.1±1.3^{Ab}	6.7±0.8^{Ac}	10.8±1.8^{A}
T7	10.3±1.8^{Ca}	7.1±1.0^{Bb}	5.1±0.5^{Bc}	7.5±1.1^{C}
Mean	11.4	7.7	5.6	8.23

Different capital letters within columns and small letters within rows are significantly different at P=0.05 according to Duncan Multiple Range Test (DMRT) for separation of means.

Table 4. Correlation coefficient (r) between SOC, MBC and specific enzyme activities across soil layers in sub humid and semi arid regions.

Variable	Sub humid region		Semi arid region	
	SOC	MBC	SOC	MBC
DHA	0.903	0.503	0.304	0.408
AS	0.663	0.651	0.542	0.614
Urease	0.067	0.183	0.786	0.764

microbial biomass, due to its larger surface area and adhesion to organic substances, helps in the formation of soil aggregates (Xiaojun *et al.* 2013). The microbes which get into earlier stages of aggregate formation would die due to unfavourable conditions, which lead to reduction in soil MBC (Schlesinger 1995). Accordingly, the soil MBC was lower in Inceptisols due to higher clay content (27.8%) than Entisols (10.6%). This has resulted in lower MBC/SOC ratios in Inceptisols over Entisols (Fig. 1a; 2a). In our study, organic, integrated and recommended dose of fertilizer recorded higher values of MQ, metabolic quotient and geometric mean of enzymes both at Entisols and Inceptisols. In this study, the proportion of MBC in SOC was affected by soil type, cropping pattern and weather conditions.

Metabolic quotient is used to estimate the ecophysiological condition of soil microorganisms (Spohn 2015). In our study, it ranged from 0.11–0.23 and 0.04–0.07 across soil layers in Entisols and Inceptisols, respectively. Lower metabolic quotient in Inceptisol than in Entisol observed in this study could be the result of relatively compact soil, poor aeration and low SOC concentration hampered the microbial proliferation in the sub-soil of the Inceptisol (Fig. 1b, 2b).

Specific enzyme activity is a good measure of soil quality than absolute enzyme activity, as it takes into account of SOC content (Trasar-Cepeda *et al.* 2008). In the present study, higher specific dehydrogenase activity was observed in Entisols than Inceptisols (Fig. 3, 4). In Entisol the treatments which included organic sources (FYM) recorded

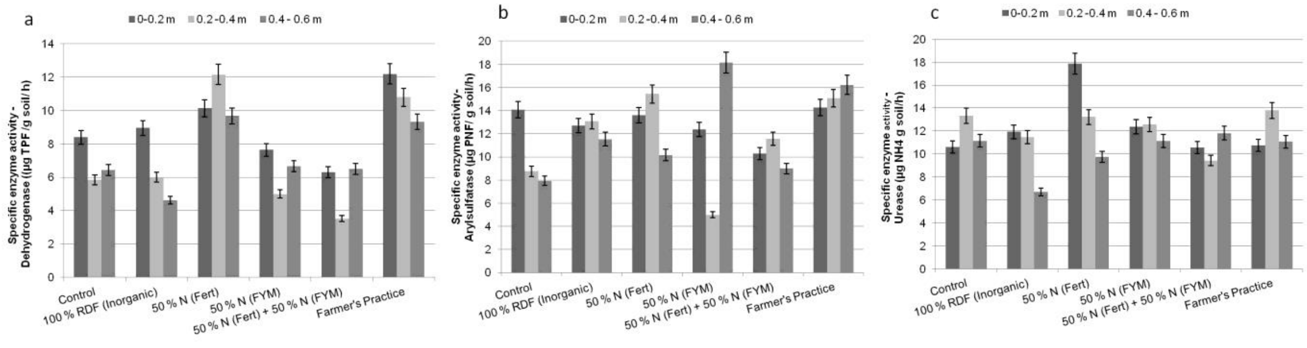

Fig. 3. Effects of long term nutrient management practices on specific enzyme activities (a) Dehydrogenase-DHA/SOC (b) Arylsulfatase (AS/SOC) (c) Urease (urease/SOC) in semi arid region Entisols of S.K. Nagar. DHA-dehydrogenase, AS-Arylsulfatase, SOC-Soil organic carbon.

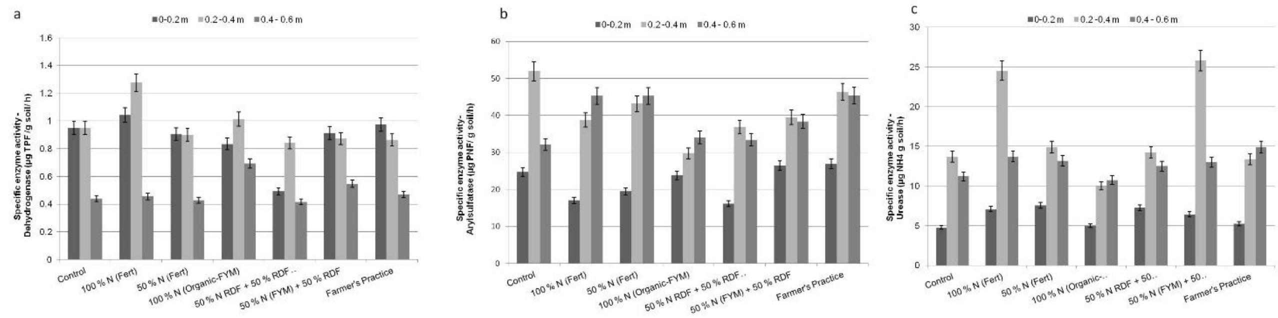

Fig. 4. Effects of long term nutrient management practices on specific enzyme activities (a) Dehydrogenase-DHA/SOC (b) Arylsulfatase (AS/SOC) (c) Urease (urease/SOC) in sub humid region Inceptisols of Varanasi. DHA-dehydrogenase, AS-Arylsulfatase, SOC-Soil organic carbon.

maximum values. Ge *et al.* (2009) reported that, in semi-arid Entisols, application of mineral fertilizers along with FYM supplies organic substrate and nutrients in readily available form that triggers microbial population and dehydrogenase activity. However, no difference in dehydrogeanse activity due to mineral or organic sources of nutrients was observed in Inceptisol. Specific enzyme activity of arylsulfatase and urease was more at Inceptisols when compared to Entisols (Fig. 3, 4). The higher specific enzyme activity indicates the presence of more enzymes per unit of carbon and loss of organic matter at a faster rate than enzyme activity (Trasar-Cepeda *et al.* 2008). The higher enzyme activity in Inceptisols may be due to presence of most labile organic matter (Raiesi & Beheshti 2014). Availability of sulfur in semi-arid Entisols of Gujarat had a negative non significant correlation with arylsulfatase. On the other hand, available sulfur was positively and significantly correlated with arylsulfatase in Inceptisols of sub-humid region of Varanasi, indicating loss of organic matter and lower organic carbon content in Inceptisols. The soil organic matter acts as an insulator of heat that protects the extracellular arylsulfatase from denaturation by high soil temperatures and large diurnal fluctuations in semi-arid tropics. Such a protective mechanism is lacking in treatments receiving only the chemical fertilizers. Release of several acids from FYM during the process of decomposition can reduce soil pH and invariably promote arylsulfatase activity. The present investigation recorded high sensitivity of urease to types and amount of amendments than to climatic-edaphic factors. In both experimental sites, long term application of FYM alone significantly reduced urease compared to the use of INM or 100% mineral fertilizers.

Geometric mean of enzymes (GMea) is an early soil quality indicator and a good index as its value is related to other soil properties (Paz-Ferreiro *et al.* 2012). Inceptisols of sub-humid region of Varanasi

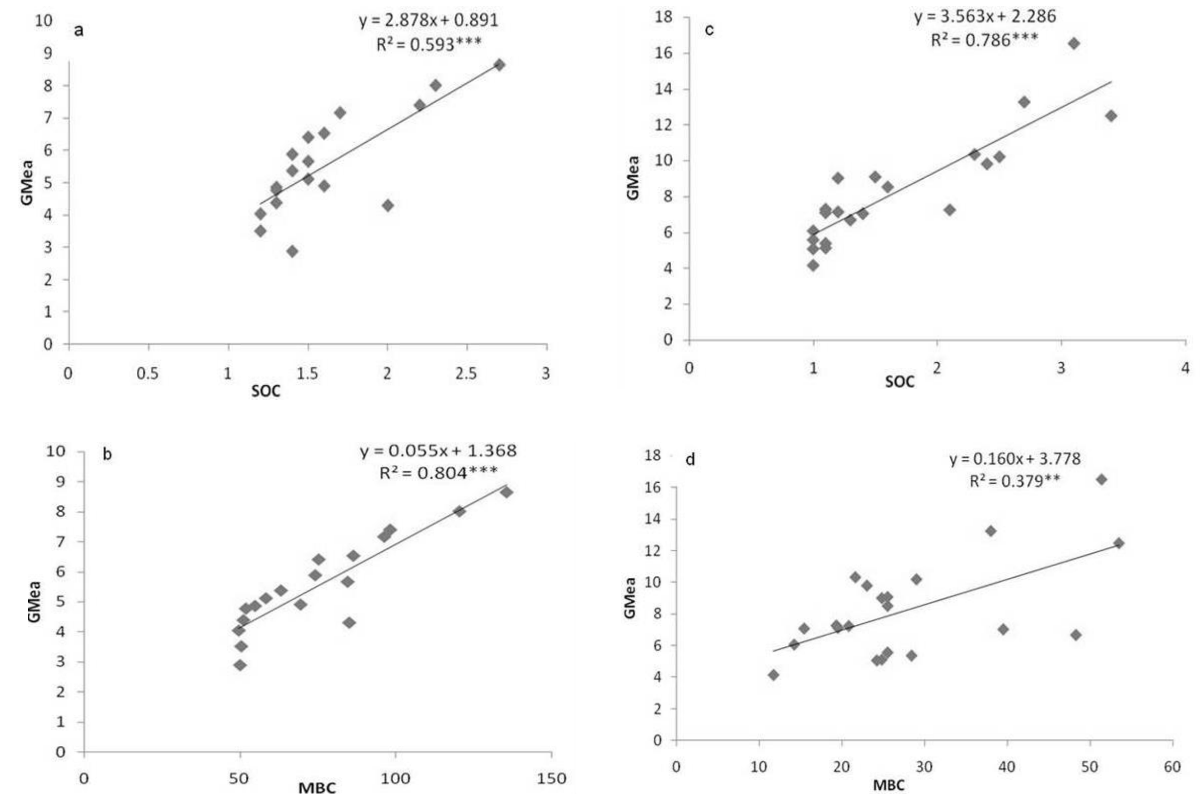

Fig. 5. Relationship of GMea with SOC and MBC in two long-term experiments: a-b: response in pearlmillet based system; c-d: response in rice based system.

with comparatively higher water availability, clay content and less fluctuations in diurnal temperature showed higher GMea than Entisols of semi-arid region of Gujarat. Inclusion of FYM with 50% reduction of mineral fertilizers significantly increased enzyme activities and GMea at both experimental sites regardless of soil type and climate.

Tillage, crop rotation, organic and inorganic fertilizers influences the level of SOC (Purakayastha *et al.* 2008). In the present study, concentration of SOC was significantly and positively correlated with enzyme activities. Soil enzymes and SOC are related to soil organic matter content, which is linked to soil health (de Medeiros *et al.* 2017).

Conclusions

Our results suggest that, use of chemical fertilizers alone lead to loss of SOC. Application of FYM and reduced dose of chemical fertilizers i.e., the application of 50% recommended dose of N through chemical fertilizer + 50% recommended dose of N through farm yard manure (N-FYM) in Entisols and 50% N-FYM + 50% RDF in Inceptisols resulted in enhancement of soil microbial activity. Long-term use of FYM in combination with mineral nutrient sources help in maintaining microbial biomass and soil organic matter, this in turn lead to sustainable soil health in both semi arid and sub humid regions of India.

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