Open Science Publications

Assessment of Enzyme Activity and Functional Microbial Diversity in Coastal and Desert Soil Ecosystems of Gujarat

Research article

Purvi Zaveri, Rushika Patel, Pooja Rana, Bhruvi Shah, Neelam Mahto and Nasreen S Munshi*

Institute of Science, Nirma University, Sarkhej-Gandhinagar Highway, Ahmedabad 382 481, Gujarat, India

*Corresponding author: Dr. Nasreen S Munshi, Institute of Science, Nirma University, Sarkhej - Gandhinagar Highway, Ahmedabad 382 481, Gujarat, India; Phone: +91-2717-241900-04, Ext.: 755, Mobile: 09898598296, Fax: +91 - 2717 - 241916 - 17; Email: nasreen.munshi@nirmauni.ac.in, nasreenhaque@hotmail.com

Abstract

All living beings of various trophic levels in ecosystem depend on the soil as a source of nutrients and depend on soil organisms to release and recycle key nutrient elements by decomposing organic residues. Microbiota of soil plays critical role in the maintenance of soil health and quality by secreting important enzymes, which are capable of function even after being released by the cell. Comparing various types of soils, salinity is profoundly observed in coastal and desert region. Study of such type of soil may give insights for understanding the variations in microbial community and effect of various abiotic factors like, salt concentrations on the same. Studying diversity at the ecosystem level is important to understand range of processes and complexity of interactions. Polyphasic approach of studying microbial community by C source utilization profiling and soil enzyme activity measurement was employed in this study to compare desert and costal ecosystems. Functional diversity was studied by CLPP method using Ecoplate®. Six different soil enzyme activities were investigated. In some samples protease, urease and L-asparaginase activity were not detected at all. β-glucosidase and L-asparaginase showed significant positive correlation with all functional diversity indices, most of the microbial groups and temperature while negatively correlated with moisture and pH respectively. Alkaline phosphatase activity negatively correlated with temperature. PCA analysis based on enzyme activity showed that samples were grouped together geographically or according to the source of origin.

Keywords: Coastal and desert soil; Community Level Physiological Profiling (CLPP); Soil enzyme activity; Principle Component Analysis (PCA)

Introduction

Soil is fundamental and irreplaceable; it governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles [1]. Members of all trophic levels in ecosystem depend on the soil as a source of nutrients and also depend on soil organisms to release and recycle key nutrient elements by decomposing organic residue [2]. Soil is a structured, heterogeneous and discontinuous system with microorganisms living in discrete microhabitats. Composition and

activity of such habitat depends on soil texture, concentrations of various salts, moisture, pH and temperature of soil. Out of various categories of soil, desert and costal ecosystems are comparable in their salinity but they differ significantly in various other factors. Microbiota of soil plays critical role in the maintenance of soil health and quality by secreting important enzymes like, degradative enzymes, which even function after being released by the cell. Such microorganisms are involved in important soil ecological functions like nutrient cycling and environmental detoxification. Each species

displays variety of morphological, physiological, and behavioural traits, many of which might influence the abundance of species and ecosystem functioning [3].

Such enormous number of organisms requires set of specific techniques to evaluate them. Studying diversity at the ecosystem level is important to understand range of processes and complexity of interactions. To reveal such complex interdependent system of microbial flora and their function, culture dependent and independent methods like, FISH, qPCR, DGGE and CLPP have been developed. Community Level Physiological Profiling has been widely used for characterizing various ecosystems like water, wastewater, various soil types and even biofilms. The contribution of microorganism in such system indirectly also is determined in terms of quantitative measurements of soil enzyme activity.

Enzyme activities are essential for energy transformation and nutrient cycling [2]. From an ecological perspective, extracellular enzymes are the mediators of decomposition, dissolved organic carbon(C) production, as well as nitrogen (N), and phosphorous (P) mineralization. According to location of the enzymes, they may be (1) Intracellular enzymes, (2)Periplasmic enzymes, (3)Enzymes attached to outer surface of cell membranes, (4) Enzymes released during cell growth and division, (5) Enzymes within non proliferating cells (spores, cysts, seeds, endospores), (6) Enzymes attaches to dead cells and cell debris, (7) Enzymes leaking from intact cells or released from lysed cells, (8) Enzymes temporarily associated in E-S complex and (10) Enzymes adsorbed to surfaces of clay minerals. Extracellular enzymes are the proximate agents of organic matter transformation in soils, however all of the above mentioned are important for normal functioning of ecological niche. Due to the effects of external disturbances on their activity, enzymes can serve as sensitive indicators of soil quality [4]. Soil enzyme activities have been related to soil physico-chemical characters, microbial community structure, vegetation, disturbance and succession.

Earlier studies have shown use of substrate utilisation profiles and enzyme activities along with the culture methods of functional groups of organisms as a Polyphasic approach to be useful for characterising coastal microbial communities [5]. Hence this study was undertaken to understand the functional diversity and their correlation with all functionally relevant enzyme activities in salt affected ecosystems. In

Table 1: Details of various soil sampling sites and collected soil and sediment samples.

Type of samples	Region/ District	Specific site of sampling	Sample label
Coastal soil	Alang	Coast	AC
		Sediment	AS
	Daman	Coast	DC
		Sediment	DS
		Polluted Coast	DPC
		Polluted Sediment	DPS
	Mandvi	Coast	MC
		Sediment	MS
	Okha	Coast	OC
		Sediment	OS
	Porbandar	Coast	PC
		Sediment	PS
	Veraval	Coast	VC
		Sediment	VS
Desert soil	Bhuj	Virgin	BV
		Near Shrub	BNS
	Dhordo	Virgin	DV
		Near Shrub	DNS
		White Rann	DWR
	Khavda	Virgin	KV
		Near Shrub	KNS
Fertile soil	Control	Botanical Garden, Nirma University, Ahmedabad	NS

India, state of Gujarat has the longest coastal belt of the country. Here coastal areas where salinity is contributed by sea water and inland desert areas where inherent salinity is present were expected to have similar microbial activities. Soil enzyme activities are considered as a measure of soil functional diversity, and are contributed by both the types of microbes, cultivable as well as uncultivable. Hence, this study is focused on the objective of determining any correlation between the enzyme activities of various salt affected lands of the state with functional diversity determined using community level physiological profiling. Among the different enzymes involved in nutrient cycling, β-glucosidase, protease, urease, cellulase, L-asparginase and phosphomonoesterase and dehydrogenase were studied [6].

Materials and Methods

Composite soil samples were collected from various coastal and desert regions of Gujarat (Table 1) [1]. Samples were stored at lower temperatures until they were processed for various physico chemical and microbial analysis. Table 1 enlists the sites of sampling and codes used here.

Determination of abiotic parameters

Various physico-chemical parameters like pH, temperature, moisture, water holding capacity, salinity, chloride, conductivity, organic carbon and total nitrogen were determined from soil and sediment samples [1,7,8].

Community level physiological profiling

Ecoplates® (Biolog® Inc., Hayward, California) containing 31 different C substrates in triplicates were inoculated with 100 µl of
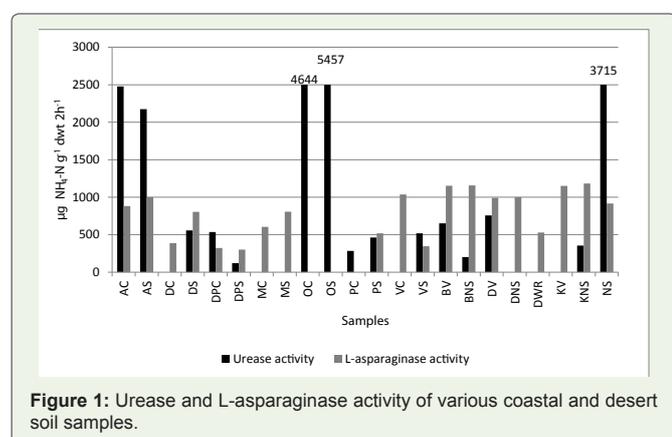
Figure 1: Urease and L-asparaginase activity of various coastal and desert soil samples.
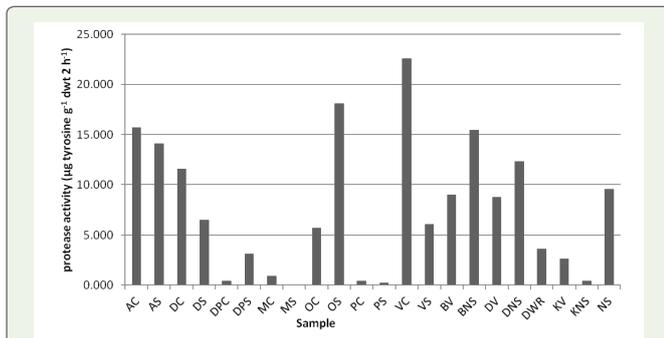
Figure 2: Protease activity of coastal and desert samples.
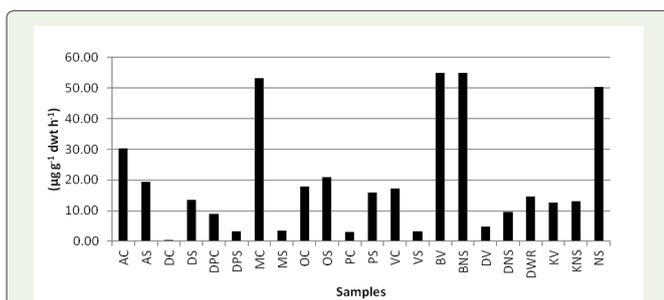
Figure 3: β-Glucosidase activity of coastal and desert samples.

appropriately diluted soil suspensions [9]. Plates were incubated at 30 ±2 ˚C. Thirty one environmentally important substrates (Carbohydrates (10), Carboxylic and acetic acid (9), Amino Acids (6), Polymer (4) and Amine/ amides (2)) were analysed for their usability by microbial communities present in the whole *soil samples*. The dye tetrazolium in wells was reduced to produce violet colour due to microbial degradative activity and colour intensity was measured spectrophotometrically at regular time intervals in a microplate reader (Bio-Rad Laboratories Inc., Elisa Plate reader Model no. 680, Japan) [10].

Various diversity indices values were calculated using collected data of average well colour development and following formula [11].

$$D_{Shannon} = H' = -\sum_i [Pi \times \ln(Pi)]$$

Where,

$$Pi = \frac{n_i}{n}$$

S = Number of species or Number of carbon substrates used in Ecoplate®.

n_i =Number of individuals of each species or Response of each C source utilization in CLPP.

n = Number of individuals in all species or sum of responses in all the wells or C substrates.

H' = Shannon diversity index.

The Margalef (1958) equation is based on the assumption that a relationship exists between S and n, total number of individual.

$$R_{Margalef} = \frac{S-1}{\ln(n)}$$

Where,

S = Number of species present or Number of C sources utilized

n = Number of individuals in all species or Sum of response of all the wells containing C sources.

The other component of species diversity indices is species evenness, which indicates the distribution of the individuals within species designations. This index group includes species abundances in its calculation. The evenness index that is most widely used is the ratio of the Shannon index [11,12]. Out of various described evenness values, E_{Pielou} was calculated using following formula,

$$E_{Pielou} = \frac{H'}{\ln S}$$

Where,

H'= Shannon-Wiener diversity value

S = Number of species present or Number of Carbon source utilized.

Quantitative measurement of various enzyme activities

Various enzymes having important functional roles in ecosystem can be categorised according to biogeochemical cycles they contribute to [6].

Table 2: Physico-chemical characteristics of soil and sediment samples.

Sample	pH	Temperature (ºC) surface subsurface		Moisture (%)	Water holding capacity (%)	Conductivity (µS)	Salinity (%)
AC	8.19	27	24	8.98	32.09	765	0.250
AS	8.3	22	ND	14.63	32.22	501	0.176
DC	8.25	25	21	21.85	28.84	1456	0.450
DS	8.4	23	20.5	18.68	23.54	1228	0.450
DPC	8.53	20	18.5	6.45	23.09	412	0.300
DPS	8.48	18	ND	16.2	40.76	824	0.300
MC	8.19	25	23	16.62	35.72	1173	0.550
MS	7.75	22.5	ND	18.88	36.23	1344	0.550
OC	8.33	21	19	7.18	34.96	844	0.350
OS	7.93	20	ND	20.75	36.46	1196	0.450
PC	8.4	21	18	11.58	41.73	719	0.400
PS	8.2	17	ND	12.8	32.45	958	0.350
VC	8.25	18	17	3.51	34.83	376	0.176
VS	8.16	18	ND	12.9	32.41	698	0.325
BV	7.84	32	25	0.26	27.83	96	0.140
BNS	8.02	29.5	27.5	0.52	30.69	56.5	0.106
DV	7.57	27	25.5	13.95	45.71	6630	0.114
DNS	7.59	27	26	14.57	49.73	5520	1.900
DWR	7.41	26	18	23.85	71.85	**41750**	7.500
KV	7.29	28.5	22	12.85	43.87	**23300**	6.850
KNS	7.82	26	25	15.1	59.51	4760	2.000
NS	ND	29	28	1.57	67.72	200	0.034
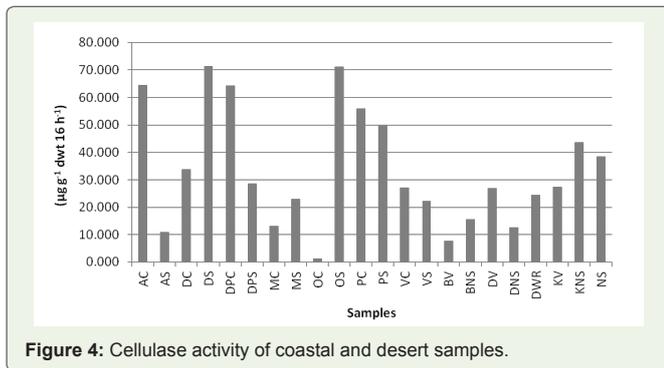
Figure 4: Cellulase activity of coastal and desert samples.
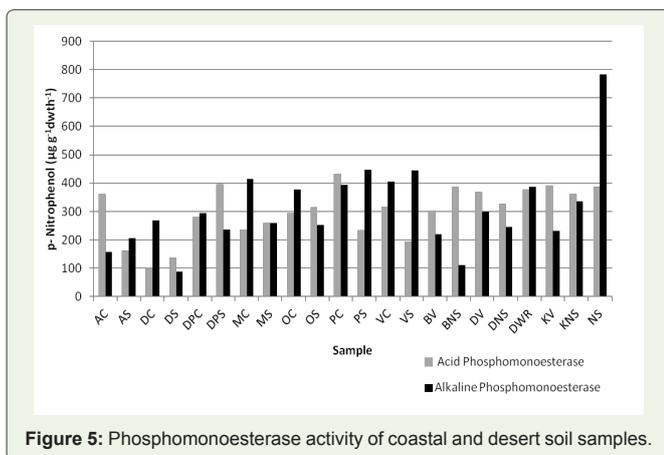
Figure 5: Phosphomonoesterase activity of coastal and desert soil samples.

Enzymes involved in Nitrogen cycling

(i)   Urease and L-asparaginase activity

For estimation of urease and L- asparaginase activity, before proceeding for actual procedure, 5 g soil was treated with 0.2 ml toluene and 9 ml of Tris buffer (50 mM, pH 9), the content was mixed properly. Treated soil samples were added with 1 ml urea or 1 ml 0.5 M L- asparagine solution and incubated for few seconds. After the incubation of 2 hour at 37˚C, approximately 35 ml of $KCl-Ag_2SO_4$ (100 mg l^{-1}) solution was added. Content was brought up to 50 ml by addition of $KCl-Ag_2SO_4$ solution and it was mixed thoroughly. The resulting solution was used to estimate released ammonia using methods described by Bremner and Keeney (1966). Calibration curve was prepared using standard ammonia solution (50 µg NH_4-N ml^{-1}) [14].

Urease activity (µg NH_4-N g^{-1} dwt $2h^{-1}$) $= \dfrac{C \times 50}{dwt \times 5}$

Where,

C = measured NH_4-N concentration (µg NH_4-N ml^{-1} soil suspension),

dwt = dry weight of 1 g moist soil

(ii)   Protease activity

It was performed using methods described by Ladd and Butler (1972). One gram of moist, sieved soil (2mm) was placed in a test tube, and 5 ml of Tris buffer (50 mM pH 8.1) and 5 ml of sodium caseinate solution (2%) were added to it. Contents were mixed and incubated for 2 h at 50 ˚C on a water bath. Five ml of TCA solution (15%) was added and mixed thoroughly. The resulting soil suspension was centrifuged at 12,000 rpm for 10 min. Five ml of the clear supernatant was mixed with 7.5 ml of the alkaline reagent and incubated for 15 min at room temperature. After addition of 5 ml of the Folin reagent, mixture was filtered through Whatman filter. Absorbance was measured after 1 h of incubation at 700 nm. Calibration curve was prepared using standard tyrosine solution 500 µg ml^{-1}. The measured absorbance was corrected for the controls and was calculated as follows,

Proteae activity (µg tyrosine g^{-1}dwt $2h^{-1}$)$= \dfrac{C \times 15}{dwt}$

Where,

C = measured tyrosine concentration (µg ml^{-1} supernatant),

dwt = dry weight of the 1 g of moist soil

Enzymes involved in Phosphorous cycling

(iii) Phosphomonoesterase has been reported to be involved in hydrolysis of organic phosphomonoester to inorganic phosphorous and makes it available for plants. Activity of this enzyme was determined by Tabatabai and Bremner (1969). One gram of moist,

Table 3: Functional Microbial Diversity indices of various soil and sediment samples collected from coastal and desert region of Gujarat.

Sample	Total activity (n)	H' Shannon	S	E_{Pielou}	$R_{Maegalef}$
AC	0.034	0.224	2	0.323	0.296
AS	0.034	0.362	2	0.523	0.296
DC	0.034	0.298	2	0.431	0.296
DS	0.043	0.309	2	0.446	0.318
DPC	0.713	0.165	3	0.150	5.912
DPS	0.377	0.309	2	0.446	1.025
MC	0.368	0.503	2	0.725	1.000
MS	0.030	0.393	2	0.567	0.285
OC	0.025	0.000	1	0.000	0.000
OS	0.039	0.637	2	0.918	0.308
PC	0.048	0.562	2	0.811	0.329
PS	0.031	0.385	2	0.555	0.288
VC	0.018	0.000	1	0.000	0.000
VS	0.041	0.494	2	0.712	0.312
BV	2.782	2.222	11	0.927	9.744
BNS	3.195	2.249	11	0.938	8.609
DV	0.049	0.506	2	0.730	0.332
DNS	0.089	0.147	2	0.213	0.413
DWR	0.042	0.300	2	0.433	0.315
KV	0.069	0.637	2	0.918	0.374
KNS	1.427	1.311	6	0.732	14.062
NS	2.182	1.947	8	0.936	8.972
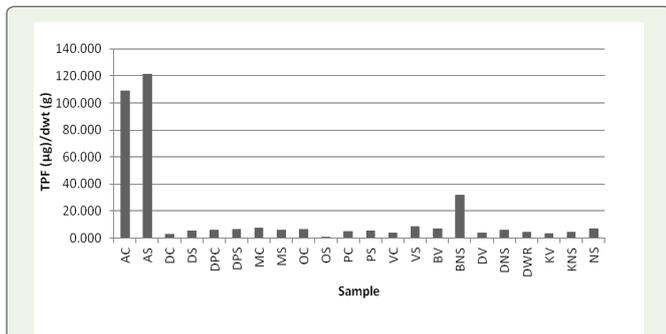
Figure 6: Dehydrogenase activity of coastal and desert soil samples.
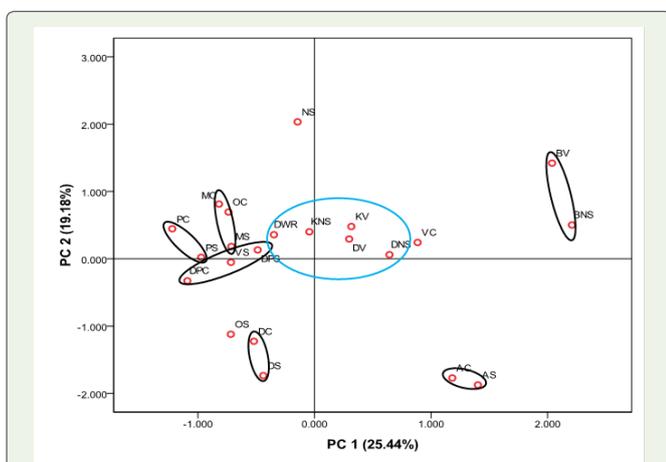
Figure 7: PCA of enzyme activity data of coastal and desert sample and clustering of samples.

sieved soil was treated with 0.25 ml toluene and 4 ml of Modified Universal buffer (pH 6.5 in assay of acid phosphatase, and pH 11) with 1 ml of p-Nitrophenyl phosphatase (15 mM) made in same buffer. The content was mixed and incubated for 1 h at 37˚C, after incubation 1ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added to the prepared mixture. The content was mixed and soil suspension was filtered through Whatman filter paper and number 2v absorbance was measured at 400 nm. P- nirophenol solution (15 mM) was used to prepare standard curve and activity was calculated using following formula. The result was corrected for the blank and was calculated according to the following relationship:

p- Nitrophenol (µg g^{-1}dwth^{-1}) $= \dfrac{C \times v}{dwt \times SW \times t}$

Where,

C= measured phenol concentration (µg phenol ml^{-1} filtrate) , v = total volume of the soil suspension in ml

dwt = dry weight of 1 g of moist soil

t = incubation period

SW = Weight of soil sample

Enzymes involved in Carbon cycling

(iv) β- Glucosidase

Activityof β- glucosidase was measured using 1 g of moist sieved soil treated with 0.25 ml of toluene and 4 ml of MUB solution. 1 ml of p- nitrophenol β-glucoside (PNG) (25 mM) was allowed to react with soil and mixture was incubated for 1 h at 37˚C. After the incubation, 1 ml of $CaCl_2$ solution (0.5 M), 4 ml of TRIS buffer (0.1 M, pH 10) were added, the flask was swirled and soil suspension was filtered immediately. Colour intensity was measured at 400 nm using UV- Visible spectrophotometer [16,17]. Calibration curve was prepared by using standard p-nitrophenol solution (25 mM). The result was corrected for the blank and was calculated according to the following relationship:

p- nitrophenol (µg g^{-1}dwth^{-1}) $= \dfrac{C \times v}{dwt \times SW \times t}$

Where,

C= measured phenol concentration (µg phenol ml^{-1} filtrate)

dwt = dry weight of 1 g of moist soil

t = incubation period

SW = Weight of soil sample

(v) Cellulase activity

For cellulase activity, 1 g of moist soil was treated with 5 ml of acetate buffer (0.1 M) and 0.5 g of Avicel were added. Tubes were incubated for 16 h at 40ºC in shaking conditions. Reaction was stopped by centrifugation (2500 g, 10 min.). One ml of supernatant was added with 1 ml of Copper reagent and were kept in a boiling water bath for 20 min. After cooling 1 ml of diluted Arsenate-molybdate solution was added and mixed thoroughly. The mixture was diluted with 3 ml of distilled water and optical density was measured at 520 nm. Calibration curve was prepared using glucose monohydrate solution (28 µg ml^{-1}). Following formula was used to determine activity.

Glucose (µg g^{-1} dwt 16 h^{-1}) $= \dfrac{C \times v}{dwt}$

Where,

C= measured glucose concentration (µg ml^{-1} supernatant)

v= volume of the soil suspension (5.5 ml)

dwt = dry weight of 1 g of moist soil

Dehydrogenase activity

(vi) Dehydrogenase activity

It was performed by methods described by Thalmann [18]. Five gram of moist soil was treated with 5 ml of Triphenyltetrazolium

Table 4: Correlation of diversity indices with abiotic parameters.

| Sr. no. | Diversity indices | Correlation with abiotic parameters | | | |
| | | Positive Correlation | | Negative Correlation | |
		Factor	r value	Factor	r value
1	H'	Temperature	(0.604)**	Moisture	(0.458)*
2	R$_{Margalef}$	Temperature	(0.521)*	-	-
3	S	Temperature	(0.604)**	Moisture	(0.563)**

Note : '*': Correlation is significant at the P<0.05 (2-tailed) '**': Correlation is significant at the P<0.01 (2-tailed),H'= Shannon diversity, R = Richness, S = Substrate Richness

Table 5: Analysis of correlation between enzyme activity and abiotic parameters.

Sr. no.	Enzymes	Correlation with abiotic parameters			
		Positive Correlation		Negative Correlation	
		Factor	*r* value	Factor	*r* value
1	β- glucosidase	Temperature	(0.589)**	Moisture	(0.622)*
2	L-asparaginase	Temperature	(0.63)**	pH	(0.482)*
3	Alkaline phosphatase	-		Temperature	(0.489)*
4	Acid phosphatase	Water holding capacity	(0.537)*	-	

Note: '*': Correlation is significant at P<0.05 (2-tailed). '**': Correlation is significant at P<0.01 (2-tailed).

chloride (TTC) (1%) and was incubated for 24 h at 30 ˚C in dark. Controls contained 5 ml of Tris buffer and treated as samples up to end of the process. After incubation, 40 ml of acetone was added to each flask and the flasks were shaken thoroughly and further incubated at room temperature for 2 h in dark. The soil suspension was then filtered in dark and the colour optical density of the clear supernatant was measured at 546 nm (red colour). Calibration curve was prepared using Triphenylformazan (500 µg TPF ml^{-1}) and results were calculated using following formula.

$$\text{Dehydrogenase activity TPF (µg)/dwt (g)} = \frac{TPF \times 45}{dwt \times 5}$$

Where,

dwt = dry weight of 1 g of moist soil

5 = moist soil used (g)

45= volume of the solution added in soil for assay

Results and Discussion

Physico-chemical analysis

Table 2 summarizes the physico-chemical characteristics of soil and sediment samples collected from various coastal and desert regions.

Highest pH was found to be in Daman Polluted coast (8.53) and lowest was found to be in Khavda Virgin soil (7.29). The coastal samples were more alkaline than dessert samples. Soil pH also affects the activity of enzymes due to the pH sensitivity of amino acid functional groups that alter conformational and chemical changes of amino acids essential for binding and catalysis. The pH can also affect enzyme activity by influencing the concentration of inhibitors or activators in the soil solution and the effective concentration of the substrate [19].

The electrical conductivity (EC) in various soil samples of coast and desert regions of Gujarat ranged between 56-41750 mS/cm. Temperature was found below 30 ºC at all the sampling sites due to winter being the season of sample collection. Significant difference between temperature of surface and subsurface of sampling sites were noted and was found that dessert samples showed higher difference than coastal samples.

Highest salinity in coastal samples was found in Mandvi region and lowest in Veraval Coast and Alang soil Sediment. In desert samples highest salinity was in soil sample of Dhordo White Rann (7.5%) and

lowest was found in soil collected from Bhuj near shrub (0.106%). The studies by Moradi et al.[20], have shown that the heterotrophic bacterial population in each soil sample was significantly less in the presence of salt. Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions. A direct correlation was seen between salinity and electrical conductivity in all soil samples ($r = 0.95**$). The highest EC was found in Dhordo White Rann (41750 mS/cm) and lowest was found in Bhuj Near Shrub sample (56.5 mS/cm).

Overall moisture content was found to be high by varying in the range of 3-24%. The moisture content of the soil causes the soil to compact. Studies had shown that the compact soil had significantly lower biomass C (38% decrease) and lower enzyme activities (decrease in range from 41-75%) than the un-compacted soil [21-23].

Microbial diversity analysis

Functional Diversity based on CLPP was first analysed by comparing AWCD (Average Well Colour Development) patterns displayed by all samples along the incubation time till 137 h (data not presented) and based on that, for calculation of diversity indices, the data of 98 h was selected. The C-source utilization by microbial communities of coastal soils was very poor as only two to three substrates were utilized by these communities, whereas among the desert soils maximum 11 substrates out of 31 were utilized by Bhuj samples.

The total activity and AWCD was obtained in the following decreasing order, BNS > KNS > DPC > MC >DNS> PC > AC > DC > OC > VC (data of AWCD not shown). Higher total activity (n), richness and H, indices indicates a greater rate of substrate utilization (catabolic potential) by the microbial community and greater functional diversity [24], however low total activity (n), substrate richness (S), diversity (H') was observed in coastal and desert samples (Table 3). Highest H' value was obtained in soil samples of Bhuj and lowest was obtained in Dhordo near shrub sample.

Table 4 presents the '*r*' values for significant correlation between diversity indices and abiotic parameters. Pearsons' correlation were calculated between diversity indices and abiotic parameters and only significant correlation values are presented. The coastal samples were having low richness as compared to desert samples, but microbial community present were able to utilize more C sources.

Table 6: Correlation of functional diversity indices with soil enzyme activity.

Sr. no.	Diversity indices	Co-relation with enzymes
		Positive Correlation
1	H'	β-glucosidase (0.856)**
		L-asparaginase (0.438)*
2	R Margelef	*β*-glucosidase (0.606)**
		L-asparaginase (0.517)*
3	S	β-glucosidase (0.897)**
		L-asparaginase (0.460)*

Note: '*': Correlation is significant at P<0.05 (2-tailed) '**': Correlation is significant at the P<0.01 (2-tailed), H' = Shannon diversity, R= Richness, S = Substrate Richness.

Diversity indices showed significant positive correlation with temperature and negative correlation with moisture. i.e. as temperature increases, H', richness and substrate utilized (S) also increase while as moisture increases H' and substrate utilized (S) decrease due to increased compactness of the soil.

Enzyme Activity

Nitrogen cycle enzymes

Nitrogen cycling is one of the most important nutrient cycles of the ecosystem. Soil becomes one of the largest return site of nitrogen due to tremendous involvement of nitrifiers and denitrifiers. Before functions of such autotrophs come in picture, there is cascade of enzyme functioning e.g. urease, asparaginase, protease etc.

(i) Urease Activity

Among various soil enzymes, urease (urea amidohydrolase, EC 3.5.1.5) is closely associated with the transformation, biological turnover and bioavailability of nitrogen and is a key enzyme [25,26]. Since the relationship between increased salinity (or/and sodicity) and reduced urease activity appears to be highly predictable, it may be used as indicator of soil quality [27].

However, coastal soil urease activity was falling in the range of 0-5457 µg NH_4-N g^{-1} dwt (Figure 1) which was found to be higher in some cases than desert region. Highest activity was observed in Okha sediment and lowest in Daman polluted sediment. In Mandvi, Veraval coast, and Daman coast soil samples urease activity was not detected at all. In desert samples urease activity was found in the range of 0-758 µg NH_4-N g^{-1} dwt $2h^{-1}$.

Less enzyme secretion in most of desert soil may be explained by the high salinity/sodicity of the soil. In addition, urease is extracellular, stable and form complexes with the organic and mineral colloids [28,29], while salinity may induce degradation in arid or semiarid soils.

(ii) L-Asparaginase Activity

In general, L-Asparaginase activity was found to be quite high in all samples except Okha and Porbandar samples where it was not detected (Figure 2). In coastal samples, L-asparaginase activity found was in the range of 300-1040 µg NH_4-N g^{-1} dwt $2h^{-1}$. Highest activity was observed in Veraval coast and lowest in Daman polluted sediment. In desert samples L-Asparaginase activity was found in the range of 345-1200 µg NH_4-N g^{-1} dwt $2h^{-1}$.

(iii) Protease Activity

Daman, Mandvi and Porbandar among coastal samples had very low protease activity while desert samples showed average activity (Figure 2). All the samples showed protease activity except Mandvi sediment.

In coastal soil samples, protease activity was found in the range of 0-23 µg tyrosine g^{-1} dwt $2h^{-1}$. In desert samples protease activity was found in the range of 0.4-16 µg tyrosine g^{-1} dwt $2h^{-1}$. Highest was observed in Bhuj near shrub and lowest in Khavda near shrub.

Carbon cycle enzymes

(i) β-Glucosidase Activity

The order of average β-glucosidase in costal soil was, MC> AC> OS> AS> OC> VC> PS> DS> DPS> VS> PC> DC (Figure 3). Analysis showed that activity in soil collected from coast of Daman region was significantly lower than other samples (Activity being 0.50 µg g^{-1}dwt h^{-1}). In desert samples, β-glucosidase activity was found in the range of 4.8-300 (µg g^{-1} dwt h^{-1}). Highest activity was observed in Bhuj samples and lowest in Dhordo virgin soil sample. The specific activity of β-glucosidase has been correlated with monounsaturated fatty acids, typical of Gram-negative bacteria [30].

The differences in the sources of substrate availability and composition may lead to the changed behaviours of the activity of hydrolytic enzymes, such as phosphomonoesterase, urease and β-glucosidase in soils. Soil organic carbon (SOC) and microbial biomass have been reported to be negatively correlated in case of wasteland soil ecosystem and β-glucosidase activity [31].

(ii) Cellulase activity

Cellulase activity was detected in all the coastal and desert samples analyzed (Figure 4). In coastal samples, cellulase activity was found in the range of 1-72 (µg g^{-1} dwt 16 h^{-1}). Highest activity was observed in OS (Okha sediment) and lowest in OC (Okha coast). In desert samples, cellulase activity was found in the range of 7-44 (µg g^{-1} dwt 16 h^{-1}).

Highest activity was observed in Khavda soil near shrub (KNS) and lowest in virgin soil of Bhuj. This may be due to the positive effect of plant organic matter present in soil near shrub. In general, coastal soils displayed higher cellulase activity as compared to desert samples. In a field study conducted in the Negev Desert over three seasons, concentration of cellulase in the soil was determined by monitoring the rate of solubilization of chromophoric molecules covalently linked to artificial insoluble cellulose (cellulose-azure). It was observed that, when cellulose of plant or paper origin was added to the study soils it was influenced by the cellulose source. It was also markedly affected by seasonal changes over the year [32].

P cycle enzymes

Phosphomonoesterase Activity

In coastal soil, alkaline phosphatase activity was found in the range of 88-443 p-nitrophenol µg g^{-1} dwt h^{-1} (Figure 5).

All the samples tested from various region displayed Phosphomonoesterase activity. Highest activity was observed in Veraval sediment and lowest in Daman sediment. As reported by Dick et. al.[21], low alkaline phosphatase activity was observed in desert samples as compared to acid phosphatase activity as the soil salinity inhibited the enzyme activity of alkaline phosphatase.

Dehydrogenase Activity

Dehydrogenase activity in soil has been used as a measure for overall microbial activity. Estimation of dehydrogenase activity

in all coastal and desert soil samples indicates the presence of microorganisms active in metabolism. Figure 6 displays the results of dehydrogenase activity of samples.

In coastal soil samples, dehydrogenase activity was found in the range of 1-121 µg Triphenylformazan (TPF) /dwt (g), where as in desert soil samples, dehydrogenase activity was found in the range of 3-31 TPF (µg)/dwt (g). Highest activity was observed in soil sample of Bhuj region collected around shrub and lowest activity was observed in Khavda soil sample unaffected by manual disturbances as well as having no plant growth.

Microorganisms growing under water-logged conditions have been shown to have lower TTC dehydrogenase activity [33]. However, only in Mandvi and Okha sediment soil, lower dehydrogenase activity than in coastal soil sample was observed. Studies have shown that dehydrogenase activity can be severely inhibited by salinity [34], hence it was assumed that dehydrogenase activity was low due to inhibition by high salinity.

Principal Component Analysis of various enzyme activity

Enzyme activities of all the samples were analysed using Principal Component Analysis (SPSS, ver. 17.0). Principle component analysis of enzyme activity data of coastal and desert samples is presented in Figure 7. On the basis of enzyme activity, soil samples were found to cluster together in 8 different groups according to regions except OS (Okha sediment). Coastal and sediment soil samples were grouped together while virgin and near shrub soil of desert samples were grouped together, except for Bhuj samples which formed a different cluster (as shown in blue colour).

Here total 3 principal components were extracted. The variance explained by PC1 was 25.44%, PC 2 was 19.18% and PC3 explained 16.02% variance present in the data.

The correlation coefficient values were calculated from enzyme activity data and various abiotic factors, where temperature was observed to be important factor affecting enzyme activity (Table 5). Pearsons' correlation values were calculated between enzyme activity and abiotic parameters for analysis of positive and negative impact of abiotic factors on enzyme functioning.

Enzyme activities of β-glucosidase and L-Asparaginase showed a significant positive correlation with temperature i.e. activity of these enzyme increases with the increase in temperature while they showed negative correlation with pH and moisture respectively i.e. activity of β-glucosidase decreases with increase in pH and activity of L-Asparaginase decreases with increase in moisture. Moisture was seen to affect microbial activity and enzyme activity and a significant negative correlation was observed in Pearsons' bivariate correlation analysis. Alkaline phosphatase showed negative correlation with temperature and acid phosphatase showered positive correlation with water holding capacity. Other enzyme activities didn't show any significant correlation with abiotic parameters.

Diversity indices showed positive correlation with most of the microbial groups (data not shown). When the enzyme activities were correlated with functional diversity indices values, significant positive correlation was found only for two enzyme assays (Table 6). Pearsons'

correlation coefficient values between functional diversity indices and soil enzyme activity were analysed and only significant correlation coefficient values are presented.

Diversity indices showed significant positive correlation with β glucosidase and L-asparaginase. When enzyme activities were correlated with quantitative values of diversity indices, very high significant correlation values (r>0.8) were observed. Such high values were not obtained in other cases. Hence it can be concluded that functional diversity deduced by CLPP could be correlated with the soil enzyme activity and can give insight of ecosystems having functionally active microbial systems.

Conclusion

Combination of two approaches, CLPP and enzyme activity quantification proved to be good tool to analyse functional microbial diversity of coastal and desert regions of the state. One of the major observations of the study indicates that although being almost near in salinity values, samples differed a lot in the functionality of the microbial flora. Abiotic factors as reported have definite correlation with enzyme activity and diversity indices. Looking to the values of diversity indices, microbial diversity was found to be low due to effect of salinity of soil. However enzyme activity and diversity indices could be correlated very well, indicating strong influence of microbial presence on nutrient cycling of such versatile ecosystem. Further analysis of community structure may help in dissecting the interactions and identification of the type of microbes but CLPP and soil enzyme activity study hold the importance for evaluation of functions of salinity affected soil systems.

References

1. Alef K and Nannipieri P (1995) Methods in Applied Soil Microbiology and Biochemistry. Academic Press Ltd., London.

2. Kandeler E (1996) Nitrate. In: Schinner F, Ohlinger R, Kandeler E, Margesin R (eds) Methods in soil biology. Springer, Berlin. 135-143.

3. Tilman D (2001) Functional Diversity. In:Encyclopedia of Biodiversity, St. Paul (ed.), vol 3, Elsevier, Germany.

4. Baldrian P (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ 55: 370-378.

5. Biswas D, Mandal A, Akbari H, Munshi N (2013) Investigation of functional microbial diversity and related abiotic factors in coastal and desert ecosystem of gujarat. In: Prospects in Bioscience: Addressing the Issues, Springer, India. 351- 362.

6. Tripathi S, Chakraborty A, Chakrabartia K, Bandyopadhyay BK (2007) Enzyme activities and microbial biomass in coastal soils of India. Soil Biol. Biochem 39: 2840-2848.

7. Forster JC (1995) Ch 3 Soil sampling, handling, storage and analysis, In: methods in applied soil microbiology and biochemistry. Alef K. and Nannipieri P. (eds) Academic press limited, London. 49-116.

8. Gupta PK (2007) Methods in environmental analysis water, soil and air. (2ndedn), Agrobios, India.

9. Preston-Mafham J, Boddy L, Randerson PF (2002) Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles - a critique. FEMS Microbiol Ecol 42: 1-14.

10. Zaveri P, Munshi N, Vaidya A, Jha S, Kumar GN (2015) Functional microbial diversity dynamics in common effluent treatment plants of South Gujarat and hydrocarbon degradation. Can J Microbiol 61: 389-397.

11. ZakJC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: A quantitative approach. Soil Biol Biochem 26: 1101-1108.

12. Pielou EC (1975) Ecological diversity. Wiley, New York.

13. Bremner JM, Keeney DR (1966) Determination and isotope-ratio analysis of different forms of nitrogen in soils: 4. Exchangeable ammonium, nitrate, and nitrite by direct-distillation methods. Soil Sci Soc Am Proc 30: 583-587.

14. Tabatabai MA, Bremner JM (1972) Assay of urease activity in soils. Soil Biol Biochem 4: 479-487.

15. Tabatabai MA and Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1: 301-307.

16. Tabatabai MA (1982) Soil enzymes. In: Methods of soil analysis part 2, chemical and microbiological properties. Page A. L., Miller E. M., Keeney D. R. (eds). American society of agronomy, Madison, WI. 903-947.

17. Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol Biochem 20: 601-606.

18. Thalmann A (1968) ZurMethodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtsch Forsch 21: 249-258.

19. Dick WA, Chenga L, Wang P (2000) Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol Biochem 32: 1915-1919.

20. Moradi A, Tahmourespour A, Hoodaji M, Khorsand F (2011) Effect of salinity on free living - diazotroph and total bacterial populations of two saline soils. African J Microbiol Res 5: 144-148.

21. Dick RP, Myrold DD, Kerle EA (1987) Microbial Biomass and Soil Enzyme Activities in Compacted and Rehabilitated Skid Trail Soils. Soil Sci Soc America J 52: 512-516.

22. Torbert HA, Wood CW (1992) Effects of soil compaction and water-filled pore space on soil microbial activity and losses. Commun. Soil Sci Plant Anal 23: 1321-1331.

23. Orchard VA, Cook FJ (2002) Relationship between soil respiration and soil moisture. Soil Biol Biochem 15: 447-453.

24. Frac M, Oszust K,Lipiec J (2012) Community level physiological profiling, characterization and microbial activity of soil amended with dairy sewage sludge. Sensors 12: 3253-3268.

25. Liang YC, Yang YF, Yang CG, Shen QR, Zhou JM, et al. (2003) Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil. Geoderma 115: 149-160.

26. Yuan L, Huang J, Yu S (1997) Responses of nitrogen and related enzyme activities to fertilization in rhizosphere of wheat. Pedosphere 7: 141-148.

27. Zhang YL, Chena LJ, Chenb XH, Tanb ML, Duan ZH, et al. (2015) Response of soil enzyme activity to long-term restoration of desertified land. Catena 133: 64-70.

28. Garcia C, Hernandez T, Costa F (1997) Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commu. Soil Sci Plant Anal 28: 123-134.

29. Tripathi S, Kumari S, Chakraborty A, Gupta A, Chakrabarti K, et al. (2006) Microbial biomass and its activities in salt-affected coastal soils. Biol Fertil Soils 42: 273-277.

30. Atlas RM, Bartha R (1993) Microbial ecology-fundamentals and applications. Benjamin-Cummings, Redwood City, CA.

31. Shao X, Yang W, Wu M (2015) Seasonal Dynamics of Soil Labile Organic Carbon and Enzyme Activities in Relation to Vegetation Types in Hangzhou Bay Tidal Flat Wetland. PLoS One :e0142677.

32. Doyle J, Pavel R, Barness G, Steinberger Y (2006) Cellulase dynamics in a desert soil. Soil Biol Biochem 38: 371-376.

33. RothG (1965) The biochemical activity of soils: a new method of assessment. Proceedings of South African Sugar Technol. Assoc. 276-285.

34. Frankerberger WT, Bingham FT (1981) Influence of salinity on soil enzyme activities. Soil Sci Soc America J 46: 1173-1177.