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Impact of organic and mineral inputs onto soil biological and metabolic activities under a long-term rice-wheat cropping system in sub-tropical Indian Inceptisols

Nirmalendu Basak[1,2]*, Ashim Datta[1,2], Tarik Mitran[2], Biswapati Mandal[2] and P.K. Mani[2]

[1]Division of Soil & Crop Management, ICAR-Central Soil Salinity Research Institute, Karnal 132 001, India
[2]Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani-741 235, India
*Corresponding Author E-mail: basaknirmalendu@gmail.com

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Abstract

Long-term use of organic and mineral inputs has an overriding impact on soil biological and metabolic activities and crop management. Farm yard manure (FYM), paddy straw (PS) and green manure (GM, *Sesbania sesban* L.) were used for 24- years old rice (*Oryza sativa* L.) -wheat (*Triticum aestivum* L.) cropping system in sub-tropical India to predict whether the screened soil biological and metabolic activities are correlated with system yield. The integrated approaches *viz*., NPK + FYM, NPK + PS and NPK + GM significantly increased both rice and wheat yield together by 67.5, 44.4 and 55.4%, respectively over control. However, for a few exceptions both soil microbial activity and metabolic activity were remarkably enhanced under integrated treatment NPK + FYM followed by NPK + PS, and NPK + GM, respectively. Among the studied attributes fluorescein diacetate hydrolyzing, dehydrogenase, β-glucosidase activity (β-glu) and microbial biomass C (C_{mic}) were screened through principal component (PCA) and discriminate analysis (DA) that explained nearly 89% of total variations of the entire data set. Among the four identified attributes, only β-glu assay value could predict system yield (R^2= 0.65). Further, estimation of β-glu activity in soil can predict other soil biological properties (R^2=0.96).

Key words

β-glucosidase activity, Connonical discriminate analysis, Organic input and Principal component analysis

Introduction

The rice-wheat cropping system occupies about 13.5 million ha in the Indo-Gangetic Plains of Southeast Asia and provides food and chief source of calories for million of people. For elevating the productivity of double grain crops, a cocktail of seed-cum-fertilizer-cum-irrigation technologies was initiated in mid sixties. However, decadal yield-trend analysis revealed that the food basket not only suffered for productivity stagnation but also depleted in inherent nutrient status and declines in quantity and quality of soil organic matter (Masto *et al*., 2008; Majumder *et al*., 2008). Additionally, inadequate nutrition and poor management practices have aggravated these problems. The associated threats of the system are: usual price hike of fertilizers, import dependency on P, K, S and micronutrient based fertilizers. So, the existing production system is becoming more fragile due to imbalance use of fertilizers, lack of suitable available resources within each target ecosystem, over exploitation of natural resources, reluctant for addressing good land care strategies (Basak *et al*., 2015a). For bridging the gap, an integrated nutrient management strategy has been conceptualized. Inorganic fertilizers and organic inputs *viz*., green manure, crop residue, farm yard manure and bio-fertilizer are the key component of this strategy (Tewatia *et al*., 2012). However, in Indo-Gangetic Plains only few studies have been addressed on the input of sole inorganic fertilizers or its integrated use with organic manure on soil biological and metabolic activities. As a consequence, impact of repeated use of management practices based on

organic and mineral inputs on soil biological and metabolic activities need to be investigated.

Crop residues such as straw have multiple fates in farming *viz*., source of nutrients and organic matter for soil, used as bedding or feeding for farm animals, or is burned as fuel for household or cellulosic ethanol production. Moreover, dumping of crop residue mainly rice is no doubt a problem in agricultural soils. In this context, incorporation of crop residue may be a viable option. Residue incorporation is postulated for increasing soil organic carbon (SOC) content and improving soil quality (Majumder *et al*., 2008; Turmel *et al*., 2015). However, addition of residues have secondary benefits *viz*., improving soil water storage, lower down moisture stress for succeeding crop, erosion control, better soil aggregation (Lafond *et al.*, 2009) with associated co-benefits of temporary immobilization of N and reduction of weed pressure and may minimize chemical fertilizer use. So, nitrogen loss is reduces by delaying nitrogen release in the environment (Roldán *et al.,* 2003). Additionally, it elevates the sinking capacity of C and N in soil for long storage. It declines gaseous or aqueous losses of carbon and nitrogen and takes part in green house gases mitigation strategy.

Here, statistical approaches *viz*., principal component and discriminate analysis (PCA and DA) were used to avoid arbitrary choice in selecting indicators and executing screening procedure, with zero level of bias from specific emphasis, on a single or group of soil biological and metabolic activities. So, objectives of the present study were to identify soil biological and metabolic activity; and to predict whether the screened soil biological and metabolic activities are correlated with system yield.

Materials and Methods

Description of field experiment : Field experiment with rice-wheat practice was started in 1986 in hot, humid subtropics of new alluvial zone of the state at the University Teaching Farm, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India. The experimental site usually receives an average annual rainfall of approximately 1480 mm and a mean annual minimum and maximum air temperatures of 12.5 and 36.2°C, respectively. Soil is under *Inceptisols* order and sandy loam in texture (hyperthermic Aeric Haplaquept according to U.S. Soil Taxonomy). Initial soil pH was 7.2, and consisted of 50, 29.5, and 20.5% of sand, silt, and clay. An oxidizable (Walkley and Black, 1934) organic carbon of 8.8 g kg^{-1}, bulk density of 1.2 Mg m^{-3}, and cation exchange capacity of 22.0 $cmol_{(P+)}$ kg^{-1} was recorded for soil at starting of the experiment. The experiment was laid out in a randomized block design with the following treatments: fallow (no cultivation since the initiation of the experiment) (T_1); control (no N-P-K fertilizers or organics), (T_2); 100% recommended dose of inorganic fertilizer (NPK), (T_3); NPK + farm yard manure (NPK + FYM), (T_4); NPK + paddy straw (NPK + PS), (T_5); and NPK + green manure (*Sesbania sesban* L.) (NPK + GM). Each treatment was replicated four times. For treatment T_3, State Agriculture Departments' recommended dose of fertilizers for rice and wheat crops at 120-60-60 and 100-60-40 of N, P_2O_5, and K_2O, respectively, were applied in the form of urea, single super phosphate and muriate of potash. Well decomposed FYM (7.5 Mg ha^{-1}), green manure (GM) (8.0 Mg ha^{-1}) and paddy straw (10.0 mg ha^{-1}) for treatment T_4, T_5 and T_6, respectively, are manually spread uniformly on the surface of specified plots (size: 8 m × 8 m) on fresh-weight basis. Then these organics were mixed thoroughly into soil using a power tiller at 2 to 5 day before puddling. Two crops, *viz*., rice (*O. sativa* L., cv IET 4094) and wheat (*T. aestivum* L., cv UP 262) were grown annually following the standard practices (Majumder *et al.,* 2008). Around 30 days old rice seedlings were transplanted in well ploughed submerged field and was continued up to grain filling stage (~3 months); rice was harvested on whole-plot basis at maturity at ground level. After the harvest of paddy, the field was ploughed thoroughly with tractor-drawn disc plow followed by harrowing and planking. Wheat crop was sown in the first fortnight of December at a distance of 20 cm between the rows. Wheat crop was generally harvested in the last week of March. The yield data of individual crop for last 24 years were also collected and equivalent rice yield (ERY) was calculated for each of the treatments for expressing yield in a common unit (Majumder *et al*., 2008) (Fig 1).

$$ERY(R\text{-}W) = \left[\frac{\text{(grain yield of wheat x unit price of wheat)}}{\text{unit price of rice}} \right] + \text{rice yield} \quad \text{.....(i)}$$

Soil sampling and analysis : Three representative field-moist soil samples were collected from each of the plots in

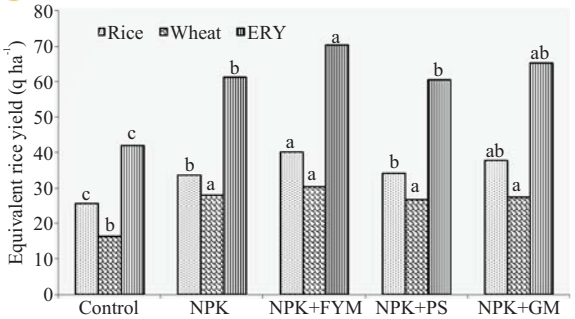

Fig. 1: Yield of rice, wheat and equivalent rice yield (ERY) of R-W system under different treatments (For the same attributes, means followed by a different letters within a row are significantly different at $P \leq 0.05$)

Table 1 : Variation in the magnitude of soil chemical, biological and metabolic activities of soils as influenced by long-term intervention

Soil attributes	Fallow	Control	NPK	NPK + FYM	NPK + PS	NPK + GM	$LSD_{0.05}$
pH_w	7.1^{d}	7.6^{bc}	7.5^{bc}	7.7a	7.6^{ab}	7.5^{c}	0.11
Cation exchange capacity	17.11^{c}	16.3^{d}	17.2^{c}	18.0b	19.6^{a}	17.6^{bc}	0.57
Organic carbon g kg^{-1}	9.9^{b}	8.2^{d}	9.3^{c}	10.9^{a}	9.9^{b}	10.2^{b}	0.38
Total N	0.71^{c}	0.75^{c}	0.81^{b}	0.91^{a}	0.79^{b}	0.83^{b}	0.04
Avail N kg ha^{-1}	133.2^{c}	136.4^{c}	151.8^{b}	162.0^{a}	153.9^{ab}	157.9^{ab}	9.2
Avail. P	38.5^{d}	64.3^{b}	72.5^{a}	61.5^{bc}	55.4c	45.7^{d}	7.3
Avail. K	216.0^{c}	247.0^{ab}	245.0^{b}	253.6^{ab}	257.0^{ab}	252.3^{ab}	12
Avail. Zn mg kg^{-1}	1.06	1.08	1.12	1.21	1.2	1.14	ns
Avail. B	0.51^{bc}	0.40^{d}	0.47^{c}	0.63^{a}	0.55^{b}	0.62^{a}	0.05
Microbial biomass C	583.0^{d}	417.3^{e}	637.4^{c}	759.3^{a}	763.5^{a}	711.9^{b}	14.5
Mineralizable C	6.99^{c}	7.28^{c}	7.43^{c}	11.63^{a}	9.84^{b}	10.75^{ab}	1.04
Mineralizable N	1.63^{bc}	1.38^{c}	1.94^{bc}	2.59^{a}	2.05^{ab}	2.19^{ab}	0.61
Dehydrogenase activity	58.0^{c}	57.9^{c}	58.7^{c}	96.7^{a}	83.4^{b}	82.3^{b}	9.7
Fluorescein diacetate hydrolyzing activity	72.7^{c}	71.5^{c}	81.7^{b}	101.3^{a}	94.3^{a}	97.5^{a}	7.9
Urease activity	45.0^{c}	54.8^{bc}	62.6^{b}	78.6^{a}	63.9^{b}	64.5^{b}	11.2
β-glucosidase activity	64.0^{c}	72.1^{bc}	80.4^{b}	106.1^{a}	100.0^{a}	99.7^{a}	10.1
Acid phosphatase activity	140.1^{cd}	133.2^{d}	156.0^{ab}	168.1^{a}	152.3^{bc}	157.4^{ab}	14.1
Alkaline phosphatase activity	129.3^{d}	252.1^{c}	276.9^{b}	307.5^{a}	286.3^{b}	312.6^{a}	19.6
Aryl sulphatase activity	88.3^{c}	113.8^{b}	121.2^{b}	146.2^{a}	144.1^{a}	125.8^{b}	12.6

[cation exchange capacity, c $mol_{(P+)}$ kg^{-1}; Microbial biomass C in µg C g^{-1}; Meralizable C in C in µg C g^{-1} d^{-1}; Mineralizable N in µg NH_4-N $g^{-1}d^{-1}$; Dehydrogenase in µg TPF g^{-1}soil $24h^{-1}$; Fluorescein diacetate hydrolyzing activity in µg fluorescein g^{-1}soil h^{-1}; Urease activity in µg NH_4-N g^{-1}soil 2 h^{-1}; β-glu, AcP, AlP and ArS are *β-glucosidase,* acid phosphatase, alkaline phosphatase and aryl sulphatase in µg *p*-nitrophenol g^{-1}soil h^{-1}; numbers followed by different uppercase letters are significantly different at $P \leq 0.05$ by Duncan's multiple-range test]

each replication from 0- to 0.2 m depth with a bucket auger on 7^{th} day after rice harvest in October, 2010. They were pooled together to make a composite sample. Samples were then hand crushed, passed through 2.0-mm sieve, and stored at 4°C, for estimating soil biological attributes. The rest portion of the field moist soil samples were dried, passed through 2-mm sieve, and used for soil chemical analysis. Soil pH in water was measured in 1: 2.5 ratio of soil : solution suspension. Cation exchange capacity (CEC) was determined following leaching exchangeable cations by neutral *N* NH_4OAc followed by removal of excess salt by 60% alcohol (Jackson, 1973). Oxidizable organic C was estimated through Walkley and Black (WBOC) (1934) wet-oxidation method. Soil available N was determined by alkaline permanganate method (Subbiah and Asija, 1956). Olsen extractant available P (soil pH > 6.0) was determined by ascorbic acid reductant method (Jackson, 1973). Exchangeable potassium (K) was extracted by neutral *N* NH_4OAc and detected by flame photometer (1973). Soil available micronutrient Fe, Mn, Zn and Cu were determined by DTPA extractable method (Lindsay and Norvell, 1978). Microbial biomass of C and N (C_{mic}, N_{mic}) were determined by Vance *et al.* (1987). C_{mic} was converted by using the relationship: C_{mic} = {(1/0.38) x C}-flush (Martens, 1995); and N_{mic} divided by a calibration factor (K_{EC}) 0.38 (Brookes *et al*., 1985). Mineralizable C (C_{min}) analysis was done through CO_2-C evolution method. The amount of CO_2 evolved during the 23 day incubation period was absorbed in 10 ml 0.5 *N* NaOH. Amount of evolved CO_2 was calculated by titrating the alkali in the traps with 0.5 *N* HCl to a phenolphthalein endpoint as outlined by Anderson, 1982. Mineralizable N (N_{min}) was estimated by aerobic incubation method (Bremmer and Keeney 1965). Ammonium and nitrate-N was extracted using 2.0 *M* KCl. NH_4 and NO_3 of the control sample were determined by following the 2.0 *M* KCl without incubating sample. Net N mineralization was estimated by subtracting the initial from final NH_4 and NO_3 content. The dehydrogenase activity (DHA) was determined by measuring pink colour intensity of triphenyl formazan (TPF) at 485 nm wavelength produced by the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) (Dick *et al*., 1996). Enzymatic hydrolysis of fluorescein diacetate (FDHA) to fluorescein was measured by extracting with acetone at 490 nm wavelength (Dick *et al*. 1996). Urease activity (URE) was determined by the NH_4 released when 5.0 g of soil was incubated with 9 ml of 0.05 *M* tris (hydroxymethyl) aminomethane (THAM) buffer (pH 9.0) and 1 ml of 0.2 *M* of urea solution at 37°C for 2 h as described by Tabatabai, 1994. The NH_4-N released was determined by steam distillation of an aliquot of the resulting soil suspension with MgO for 4 mins. β-glucosidase (β-glu) activity was analyzed through enzymatic hydrolysis of β-glucopyranoside to *p*-nitrophenol and extracted by $CaCl_2$-NaOH solution. The intensity of yellow colour of *p*-nitrophenol was measured at 410 nm

Table 2 : Principal component analysis

Statistic	PC-1	PC-2	PC-3	PC-4	Extraction
Eighen value	11.49	1.99	1.50	1.03	
% of Variance	63.83	11.06	8.34	5.70	
Cumulative %	63.83	74.89	83.23	88.93	
Eighen vectors					
Cation exchange capacity	0.66	-0.48	-0.13	0.34	0.89
Urease activity	0.77	0.22	**0.80**	0.33	0.80
pH	0.42	-0.49	0.20	0.31	0.98
Avail. P	-0.52	0.40	0.08	0.12	0.78
Mineralizable N	0.79	-0.06	-0.13	0.11	0.93
β-glucosidase activity	**0.96**	0.51	0.00	0.10	0.80
Acid phosphatase activity	0.72	-0.20	-0.11	0.09	0.95
Avail. N	0.87	-0.09	0.22	0.08	0.96
Avail. B	**0.94**	-0.12	-0.10	0.08	0.96
Mineralizable C	**0.94**	-0.14	0.29	0.05	0.92
Alkaline phosphatase activity	0.83	0.17	-0.13	0.05	0.72
Fluorescein diacetate hydrolyzing activity	**0.92**	0.05	0.34	0.01	0.91
Aryl sulphatase activity	0.81	0.03	-0.25	-0.01	0.90
Organic carbon	**0.98**	0.47	0.08	-0.11	0.92
Avail. Zn	0.61	-0.07	-0.14	-0.13	0.94
Microbial biomass C	**0.95**	-0.10	-0.03	-0.20	0.91
Dehydrogenase activity	**0.92**	**0.73**	0.61	-0.32	0.88
Avail. K	0.39	-0.38	0.17	**-0.69**	0.86

Bold figures indicate highly weighted variables among the respective PC

Table 3 : Connonical discriminant analysis

Statistic	DF-1	DF-2	DF-3	DF-4
Eigenvalue	981.77	38.82	3.43	1.88
% of Variance	95.70	3.78	0.33	0.18
Cumulative %	95.70	99.48	99.82	100.00
Canonical Correlation	1.00	0.99	0.88	0.81
Variables and discriminant coeffi cient				
Fluorescein diacetate hydrolyzing activity	**-0.638**	0.37	0.29	0.61
Microbial biomass C	-0.04	0.28	**0.743**	0.61
β-glucosidase activity	-0.03	0.24	0.27	**0.933**
Urease activity	-0.31	0.25	-0.05	0.916
Avail. B	-0.08	0.33	0.33	0.881
Mineralizable C	-0.04	0.33	0.38	0.862
Organic carbon	-0.06	0.54	0.31	0.782
Dehydrogenase activity	0.04	**0.56**	0.49	0.661

Bold figures indicate highly weighted variables among the respective DF

wavelength (Dick *et al*., 1996). Acid (AcP, pH 6.5) and alkaline (AlP, pH 11.0) phosphatase assay were analysed through phosphatase enzymatic hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol. Intensity of yellow colour of *p*-nitrophenol was measured at 410 nm wavelength. Aryl sulphatase (ArS) activity was measured based on the determination of *p*-nitrophenol released after the incubation of soil with *p*-nitrophenyl phosphate for 1 hour at 37°C (Dick *et al.,* 1996).

Screening soil attributes : At first, soil attributes were categorized according to principal component analysis. General rules of principal component were followed; that is, principal components (PC) receiving high eigen values (>1.00) and variables with high factor loadings with such components and are retained for screening. In each PC, only highly weighted factors, *i.e*., those with absolute values within 10% of the highest weight were screened for minimum data set. Further connonical discriminant analysis (CDA)

Table 4 : Regression equations using MDS variables as independent and equivalent rice yield (ERY) as dependent variable

Indicators	Unstandardized coefficients		t (df=15)	Significance level	Model
	β	Std. error			
Constant	-4.601	23.581	-0.195	0.849	Full (R^2=0.65)
Fluorescein diacetate hydrolyzing activity	0.186	0.355	0.525	0.611	
Dehydrogenase activity	-0.104	0.282	-0.369	0.720	
Microbial biomass C	0.044	0.067	0.647	0.532	
β-glucosidase activity	0.271	0.412	0.658	0.525	
Constant	12.97	10.317	1.257	0.231	Step wise (R^2=0.62)
β-glucosidase activity	0.502	0.109	4.616	0.0001	
Constant	-9.995	17.008	-0.588	0.569	Full (R^2=0.96)
Fluorescein diacetate hydrolyzing activity	0.560	0.197	2.840	0.016	
Dehydrogenase activity	0.332	0.180	1.841	0.093	
Microbial biomass C	0.039	0.048	0.816	0.432	

was employed to choose the best discriminated variables (Yao *et al*., 2013). For validation of the result, a regression analysis was done, where ERY as dependent variable and soil properties as independent variables were investigated to know whether minimum data set indicators were truly correlated with the system yield. For statistical analysis of data (PCA, CDA, regression equations) Microsoft Excel and SPSS window version 17.0 (SPSS Inc., Chicago, USA) packages were used.

Results and Discussion

The pH of soil under different treatments was almost neutral ranging from 7.1 to 7.7 (Table 1). There were little changes in pH value even after continued addition of fertilizer. All the organic amended soils showed higher cation exchange capacity as compared to those in control and fallow. Further, among the organics only FYM contained 10 and 33% more soil organic carbon (SOC) as compared to fallow and control, respectively. The highest value of available nitrogen and phosphorous was associated in soil where inorganic fertilizers were partly substituted with FYM (162.0 and 72.4 kg ha^{-1}); whereas lowest value (133.2 and 39.4 kg ha^{-1}) was observed in fallow treatment. The highest available nitrogen content in FYM treated soil was probably due to decomposition of FYM and mineralization of some of organic nitrogen. Organic matter on decomposition released organic acids which increased phosphorous availability by blocking phosphorous adsorption sites in soil or through anion exchange phenomenon. Results also showed that partial substitution of inorganic nitrogen through paddy straw and FYM was effective in increasing the available potassium content over control and NPK application. Integrated nutrient management may restore available zinc supplement in soil but external boron addition appeared a promising need for balance crop nutrition.

The amount of microbial biomass C (C_{mic}) in soil varied from 417.3 to 763.2 µg C g^{-1} soil; the relative magnitude under different treatments being as: NPK + organics> NPK> fallow> control (Table 1). Among the applied organic amendments, a maximum increase of C_{mic} was quantified in PS as compared to FYM or GM, which might be due to the presence of decomposition resistant fiber fractions (e.g. lignin, polyphenol) in PS as compared to FYM or GM (Majumder *et al*., 2008). Next to PS, presence of readily metabolisable carbon and nitrogen in organic manure increased C_{mic} in NPK+FYM**.** Further, better crop growth has a major impact on increasing root biomass which support higher C_{mic} in plots under NPK+FYM treatments (Masto *et al*., 2008). In contrast, non supply of external inputs and limited crop growth for control and sustaining bare fallow in 'fallow' treatment might hinder proliferation of microbial load. NPK + organic treatments (FYM, PS and GM) maintained a higher proportion of soil organic carbon (2.20, 2.33 and 2.2%, respectively, data not presented) in the form of mineralizable C (C_{min}) indicating higher activity of microorganisms in soil under these three treatments. However, a lower rate of CO_2 production in soil under NPK + PS incubation might be due to existence of more recalcitrant compounds such as cellulose and lignin (Thuries *et al*., 2002). Like C_{mic}, C-mineralization rate was slow in control and fallow treatments. Integrated nutrient management has potential to supply nitrogen in more adequate level compared to NPK. However, among the integrated supplementation, PS did not increase the mineralizable N (N_{min}), from NPK. This indicated that residues of PS were not easily decomposable and less suitable to promote nitrogen mineralization. But, incorporation of green manure can be prime nitrogen supplier for crop. However, an appreciable amount of nitrogen supply was visualized when FYM was integrated with NPK. Potential of nitrogen supply drastically declined when no external nitrogen was supplied. Uncultivated fallow also suffered from nitrogen starvation.

The potential metabolic activities of soil can go through the assay values of soil enzymes (Table 1). Dehydrogenase and fluorescein diacetate hydrolyzing activity (DHA and FDHA) reflects the metabolic activity of soil. DHA is present in enzyme systems of all microorganisms, and play an essential role in the initial stages of oxidation of soil organic matter by transferring electrons or hydrogen from substrate to acceptors. FDHA is a sensitive indicator of ecosystem activity; its disturbance reflects protease, lipase and esterase activities of soil (Tripathi *et al*., 2006). Both DHA and FDHA activity were significantly higher in NPK + organic treated plots as compared to soil NPK, control and fallow. However among the integrated treatments, it was significantly higher for NPK + FYM as compared to NPK + GM/PS. A strong correlation existed between enzyme activity and bioavailability of soil carbon and nutrients (nitrogen/ phosphorous/ sulphur) (German *et al*., 2011). So, assay analysis could predict some potential idea about ecosystem nutrient cycling flux (Nannipieri *et al*., 2012; Alison and Vitousek, 2005). β-glucosidase (β-glu) reflects degradation of cellulose to glucose; urease catalyzes urea into ammonia; acid and alkaline phosphatase (AcP / AlP) mineralize organic phosphorous phosphate by hydrolyzing phosphoric (mono) ester bonds under acidic/alkaline conditions; aryl sulphatase catalyses the hydrolysis of ester sulphates, and release organically-bound sulphate into soil solution. Urease activity for NPK + FYM treated plots was higher compared to other integrated plot (NPK + PS/ GM) and NPK treatment. Abundance of cellulose in soil at integrated treated plots visualized higher β-glu activity (Acosta-Martínez *et al*., 2008). As pH of soil lies within neutral range (7.1 to 7.6), it might be expected that alkaline phosphatase activity was dominant over acid phosphatase in these soil. Similarly, a 2-fold higher value of alkaline phosphatase as compared to acid phosphatase was reported in inorganic and organic amended soils (Saha *et al*., 2008). Aryl sulphatase and alkaline phosphatase activity of soil was significantly more visualised through organic amendment application rather than acid phosphatase.

The effect of 24 years of different managements on soil chemical, microbial and metabolic activity was evaluated by screening most ecosystem functioning indicators. 18 most valuable soil attributes were considered. A double screening procedure *viz*., principal component analysis and canonical discriminate analysis were used for proper identification of soil indicators. Four principal components were developed through PCA analysis. Among them first three were considered for screening (Table 2). Only 5.7 of total variance of data were described in PC-4 and eighen value of only 1.0 appeared for PC-4 (Norman and Streiner, 2008). Available potassium was highest loaded attribute in PC-4. Moreover, soil of studied ecosystem did not suffer from potassium deficiency. So, PC-4 was entirely excluded from minimum data set. The highest loading attributes under PC-1 was SOC. A 10% loading of SOC under PC-1 covered another six attributes (β-glu, C_{mic}, C_{min}, available B, DHA and AlP). PC-2 explained 11% of total data set with reweighting DHA. The importance of dehydrogenase activity as soil microbiological attributes has been emphasised by earlier (Basak *et al*., 2015b). Chemical attributes, CEC appeared as the highest loading attributes from PC-3. Overall data set of PCA revealed that, share of total data set (64%) was explained from PC-1 with 7 numbers of soil attributes. Further, discriminate analysis (DA) was employed with the screened attributes to choose the best discriminated variables. Four segregated DFs, explaining ~95.0, 3.0, 0.5 and 0.3% of the total variance with canonical correlation of 1.00, 0.99, 0.88, and 0.81, respectively were generated (Table 3). In DF-1, FDHA was more powerful co-efficient as it covered highest factor loading (-0.64). Likely, DHA, C_{mic}, and β-glu had high co-efficient (0.56, 0.74 and 0.93) in DF-2, 3 and 4 that revealed a clear indication to include in minimum data set.

Regression analyses both entire and stepwise were performed. Equivalent rice yield (ERY) was selected as dependent variable and selected soil properties were interpreted as independent variables to validate whether the soil attributes in minimum data set were correlated with ERY. The results (Table 4) showed that only β-glu ($P < 0.0001$) significantly predicted ERY ($R^2 = 0.62$). Additionally, it appeared that assay value of β-glu could predict relative soil biological status as it predicted FDHA, DHA and C_{mic} status ($R^2 = 0.96$). So, β-glu activity could be used as a good indicator for assessing soil quality and ecosystem function and metabolic activity. Activity of β-glucosidase (β-glu) could express degradation of cellulose to glucose and bioavailability of C-supply in soil according to the soil management practices (Cañizares *et al*., 2011). In a similar long-term experiment with application of organic amendment, described the activity β-glu for representing the changes in soil metabolic activity (Stott et al., 2013; Martín-Lammerding *et al*., 2015). The applied statistical approaches not only screened the minimum data set of attributes from a large of data pool but also account for ~90% variability of total data set. It needs not to say that the selection of data set through double statistical approaches can predict system yield. Moreover, the applied statistics were free from arbitrary and biased selection of soil attributes.

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