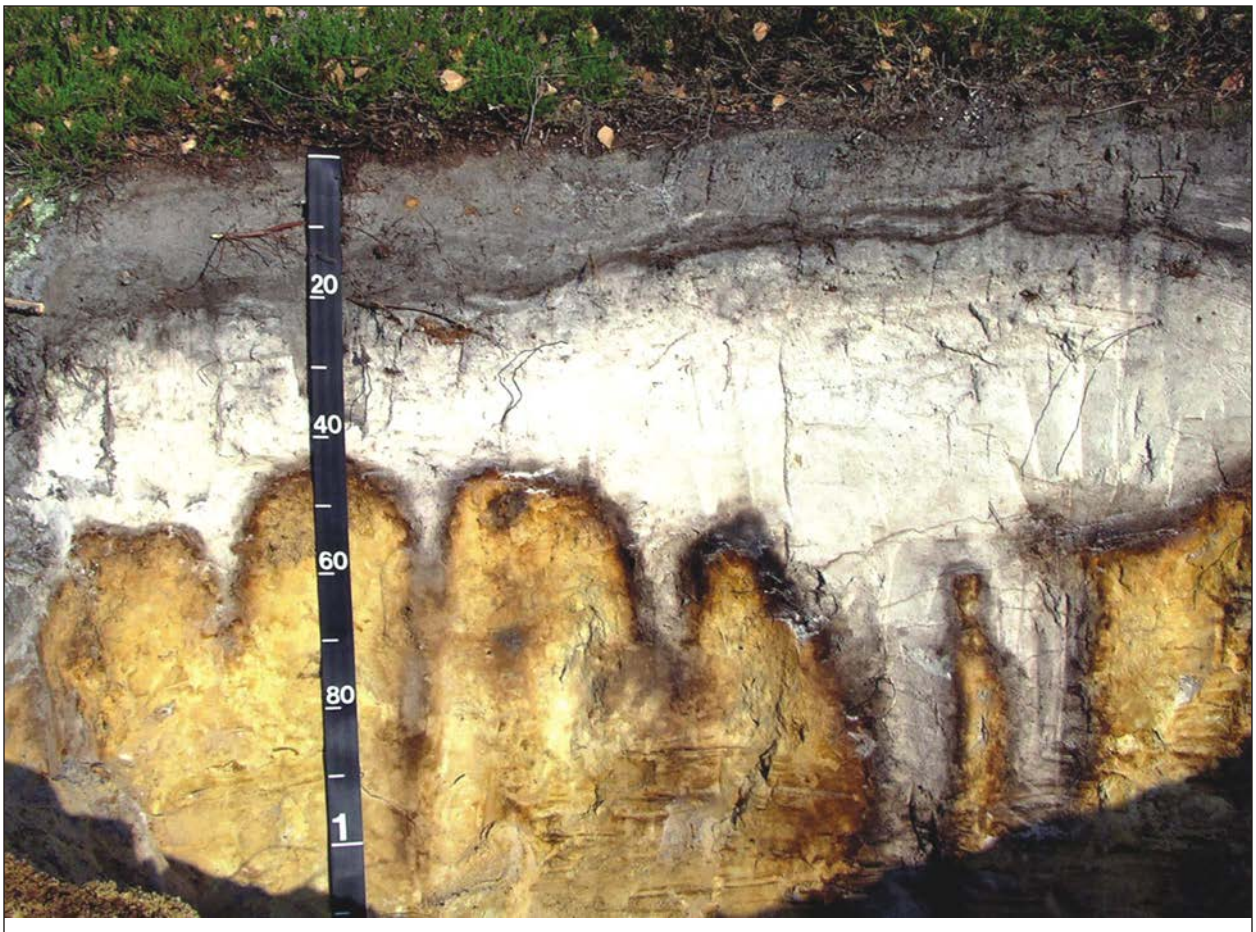

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Soil Enzymes Activities in Irrigated and Rain-Fed Vertisols of the Semi-Arid Tropics of Sudan*

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Abstract: Soil management practices that involve intensive traditional ploughing and disking may affect soil quality. Soil enzymes activities were investigated from crop rotations in irrigated and rain-fed areas. Soil samples collected from long term (79 years), medium-term (46 years) and short-term (22 years) irrigated cotton (*Gossypium hirsutum*) schemes and rainfed cultivation of sorghum (*Sorghum bicolor*) and sesame (*Sesamum indicum*) in a semi-arid tropical Vertisol. Alkaline phosphatase was significantly higher in both short-term (661 μg p-nitrophenol g^{-1} soil h^{-1}) and rain-fed cultivation (605-747 μg p-nitrophenol g^{-1} soil h^{-1}). Long- and medium-term cultivation in the irrigated sector had significantly less protease activity [3.75-4.73 μg tyrosine g^{-1} soil (2 h^{-1})] compared to other cultivation systems [11.54-15.09 μg tyrosine g^{-1} soil (2 h^{-1})]. Except, long-term cultivation, there was a general separation in the activity of β-glucosidase between irrigated [average of 21.9 μg saligenin g^{-1} soil $(3\ h)^{-1}$] and rainfed Vertisols [17.9 μg saligenin g^{-1} soil $(3\ h)^{-1}$]. Correlation analysis and Principal Component Analysis (PCA) revealed that only alkaline phosphatase activity was positively correlated with total soil N and carbon contents. These results may draw attention on the impact of intensive application of agro-chemicals (pesticides, herbicides and fertilizers) on soil health in the world biggest Gezira cotton scheme.

Key words: Crop rotation, long-term cultivation, soil management, soil quality, tillage system

INTRODUCTION

Soil organisms and their activities contribute a wide range of essential services to the sustainable function of all ecosystems. Soil enzymes are important for catalyzing innumerable reactions necessary for life processes of microorganisms in soils, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure (Dick, 1994). Although, enzymes are primarily of microbial origin, they can also originate from plants and animals. These enzymes are constantly being synthesized, could be accumulated, inactivated and/or decomposed in the soil, assuming like this, great importance for the agriculture for their role in the recycling of the nutrients (Tabatabai, 1994; Dick, 1997). Soil enzyme activities

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have been earlier discriminated between wide ranges of soil management practices (Gupta and Germida, 1988; Dick, 1997). Gupta and Germida (1988) showed that intensive cultivation can cause decreases in microbial biomass and its activity.

Although, there is ample data showing the relation between soil management and soil enzymes activities, very little is known about their performance under tropical/subtropical and arid conditions (Dick, 1984; Dick *et al*., 1988; Deng and Tabatabai, 1996). Agriculture and livestock were the main sources of livelihood in Sudan where 61% of the working population are involved and produce about 90% of the national food requirement. Crop cultivation is divided between a modern, market-oriented sector comprising mechanized, large-scale irrigated and rainfed farming (mainly in central Sudan) and small-scale farming following traditional practices carried in other parts of the country where rainfall or other water sources were sufficient for cultivation. Large investments continued to be made in the 1980s in mechanized, irrigated and rainfed cultivation, with their combined areas accounting for roughly two-thirds of Sudan's cultivated land in the late 1980s. Although, cotton remained the most important crop, groundnuts, wheat and sugarcane had become major crops and considerable quantities of sesame were also grown. Agriculturally, the most important soils are the clays of the central (also known as black cotton soils). These soils constitute the main soil type in the irrigated Gezira, Rahad and Managil schemes.

Recently, ecological assessment of ecosystems is a priority that helps land managers and policy makers to promote long-term sustainability, yet quantifying environmental sustainability remains an elusive goal (Herrick, 2000; Hurni, 2000; Bourma, 2002; Von-Wirén-Lehr, 2001). One approach is to use soils as indicators of ecosystem health (Doran and Zeiss, 2000; Sherwood and Uphoff, 2000). Historically, chemical and physical soil properties have been used as crude parameters of soil fertility and productivity. The content of soil C and N has been related to soil tilth. However, C and N changes are slow to be a useful ecological indicator (Körschens and Weigel, 1998; Schulz, 2004). There is growing evidence that soil biological parameters could be found by monitoring responses of the microbial community as early and sensitive indicators of soil fertility, ecological stress or restoration processes (Waksman, 1922; Pascual *et al*., 2000; Filip, 2002; Bending *et al*., 2004). Many enzyme assays are simple to run and sensitive to changes in management practices (Freeland, 1977).

In the semi-arid tropics of Sudananese Vertisols, some authors (Dawelbeit and Babiker, 1997; Salih *et al*., 1998; El-Awad, 2000; Mubarak *et al*., 2005) studied the impact of tillage or cultivation on water conservation, crop yield and soil physical properties, others (Buraymah and Webster, 1989) concentrated their study on the evaluation of topsoil (0-30 cm) pH, electrical conductivity or sodium adsorption ratio. There is a need, to estimate the effects of long-term cultivation of irrigated and rain-fed areas on other soil quality parameters. Therefore, the aim of this study was to determine soil enzyme (alkaline phosphates, β-glucosidase and protease) activities under different cultivation periods of an irrigated and rain-fed Vertisols.

MATERIALS AND METHODS

Study Site and Land Use Systems

This study is part of a research project aimed at determination of long-term effects of cultivation on soil quality executed between April, 2004 and April, 2008. Three study sites from the irrigated Vertisols and one site from the rain-fed areas of the semi-arid region of Sudan with different duration of cropping history were selected for this study. The irrigated Vertisols were represented by Gezira Rotation (GR) ("lat" 14° 24′ N; "long" 33° 30′ E; "alt"

390 m above MSL), Managil Rotation (MR) ("lat" 14° 14′ N; "long" 32° 49′ E; "alt" 390 above MSL) and Rahad Rotation (RR) ("lat" 13° 43′-14° 35′ N; "long" 34° 22′ - 35° 55′ E; "alt" 600 above MSL). The area of Eastern Rahad was selected to represent the rain-fed area, with two sets of rotations: viz: Sorghum-Sesame (SS) (*Sorghum bicolor* L. and *Sesamum indicum* L.) and Continuous Sorghum (CS). According to Soil Survey Staff (1996), these soils were classified as a fine, smectitic, isohyperthermic, enticchromustert (Table 1). The rotation system in the studied schemes encountered various changes according to recommendations of the National Research Corporation (Table 2). Accordingly, Gezira stared in 1925 with a three course rotation system and ended with five course rotation system. The Managil scheme (extension of Gezira) started in 1958 but however, ended with similar rotation system to Gezira. Both schemes contained rotations that fulfilled the requirements of farmers and inclusion of cash crops as well. However, the rotation system in Rahad scheme assumes ground nut as the main legume that improves soil fertility. In all schemes, similar crops have similar cultural practices (Table 3). Main crops include cotton (*Gossypium barbadense* L.), sorghum (*Sorghum bicolor* L.), groundnut (*Arachis hypogaea* L.) and wheat

Table 1: Physico-chemical topsoil (0-30 cm) properties of the seven investigated sites

Sites	C/N	N_{tot} ------ (g kg^{-1}) ------	C_{org}	R_{mean} (mm)	Water supply	T_{max} ----- (°C) -----	T_{min}	Sand -------- (g kg^{-1}) --------	Silt	Clay
PF	4.06	0.36d	1.46f	200-450	Irrigated	40	13	16	30	54c
GR	10.64	0.33d	3.51e	200-450	Irrigated	40	13	16	31	53c
MR	11.19	0.48c	5.37d	200-450	Irrigated	40	13	22	20	58b
RR	12.17	0.59b	7.18b	350-650	Irrigated	35	10	18	22	60b
NF	10.14	0.64a	6.49c	350-650	Rainfed	35	10	15	20	65a
SS	11.55	0.67a	7.74b	350-650	Rainfed	35	10	17	19	64a
CS	13.53	0.62a	8.39a	350-650	Rainfed	35	10	19	21	60b

GR: Gezira natural grass, GR: Gezira rotation, MR: Managil rotation, RR: Rahad rotation, RF: Rahad natural forest, SS: Rahad sorghum-sesame, CS: Rahad continuous sorghum, PF: Permanent fallow. N_{tot}: Total N, C_{org}: Organic C, R_{mean}: Mean annual rainfall, T_{max}: Maximum annual temperature, T_{min}: Minimum annual temperature. Values in columns followed by the same letter(s) are not significantly different at the 0.05 level

Table 2: Historical tillage background of the three investigated rotation sites

Sites	Cropping sequence
Gezira (GR)	
1925-1931	C - S/L - Fa
1932-1933	C - Fa - Fa
1934-1960	C - Fa - Fa - C - Fa - S - L/Fa - Fa
1961-1974	C - W - Fa - C - Gn/L - S - P - Fa - Fa
1975-1986	C - W - Gn/S/Veg - Fa
1987-1990	C - W - Gn/S/Veg - Fod - Fa
1991- to date	C - S - Gn - W - Fa
Managil (MR)	
1958-1960	C - Fa - C - Gn/S/L - S - Fa
1961-1975	C - W - Gn/S
1976-1988	C - W - Gn/S/Veg - Fa
1989-1990	C - W - Gn/S/Veg - Fod - Fa
1991- to date	C - S - Gn - W - Fa
Rahad (RR)	
1982-1990	C - Gn
1990- to date	C - Gn - S/W

C: Cotton, S: Sorghum, L: Legume, Fa: Fallow, W: Wheat, Gn: Groundnut, P: Philipisara, Veg: Vegetable, Fod: Fodder crop

Table 3: Crop tillage and fertilization

Crops	Tillage and fertilization
C	Disc ploughing (18-20 cm), harrowing (10-15 cm), ridging, green ridging, 120 kg N ha^{-1}
W	Disc harrowing (6-7 cm), levelling, 80 kg N ha^{-1}, 90 kg TSP ha^{-1}
S	Disc harraowing (15-17 cm), ridging, 80 kg N ha^{-1}
Gn	Disc harrowing (15 cm), ridging, green ridging

C: Cotton, S: Sorghum, W: Wheat, Gn: Groundnut

(*Triticum aestivum* L.). For control sites of the irrigated Vertisols, samples were collected from a Permanent Fallow (PF) plot of C4 grasses (mainly *Cynodon dactylon* L.). This plot is neither fertilized nor grazed (since 1937). However, for the rain-fed area, a Natural Forest (NF) of mainly *Acacia* sp. was selected.

Soil Sampling and Analysis

A field of 90 ha in both irrigated and rain-fed areas was targeted for sampling. Each field was divided into three areas (30 ha each) and three profiles (i.e., three replicates) were dug (i.e., one profile each 30 ha) to the depth of 90 cm. Samples were carefully collected using a tray to represent the entire topsoil layer (0-30 cm). Similarly, for the control of the irrigated and rain-fed areas, three replicate profiles were also dug (i.e., three in irrigated permanent fallow and similar three profiles in the natural forest). The total number of samples for the entire study was 21. Samples were placed inside a cotton bag and transferred to the laboratory, air-dried and sieved through a 2 mm sieve to remove fine roots and gravels.

Both total soil carbon (C_{tot}) and nitrogen content (N_{tot}) were determined by the Institute of Soil Science and Soil Conservation, Justus Liebig University, Germany using a combustion analyzer (Vario EL III, CHNOS, Elementar, Germany). Soil inorganic carbon (carbonate-C) was determined by the pressure transducer method adapted from Loeppert and Suraez (1996) assuming 12% C in $CaCO_3$. Organic carbon (C_{org}) was determined by subtracting inorganic C from values of total C (McLean, 1982).

Enzyme Assays

Before microbiological analyses, the air dried soil samples were moistened to 50% of water holding capacity and equilibrated to room temperature for 10 days. The activity of alkaline phosphatases (EC 3.1.3.1) was assayed using 1 g moistened soil, 4 mL modified universal buffer (pH 11), 0.25 mL toluene and 1 mL 25 mM p-nitrophenyl phosphate (Tabatabai and Bremner, 1969). The activity was determined after incubation for 1 h at 37°C by measuring the absorbance at 400 nm (Spetrophotometer U-2000, Hitachi Ltd., Tokyo/Japan) of the p-nitrophenol [p-NP] released and expressed in μg p-NP g^{-1} soil h^{-1}.

The assay of the activity of proteases (EC 3.4.2.21-24) was based on that of Ladd and Burtler (1972). Moistened soil (1 g oven-dry equivalent) was incubated with 5 mL of 50 mM tris buffer (pH 8.1) and 5 mL of 2% Na-caseinate at 50°C for 2 h; enzyme activity was then stopped by the addition of trichloroacetic acid (0.92 M). The aromatic amino acids released were measured colorimetrically using Folin-Ciocalteu reagent at 700 nm, with tyrosine as standard. The activity was expressed in μg tyrosine g^{-1} soil $(2\ h)^{-1}$.

Determination of the activity of β-glucosidases (EC 3.2.1.21) was based on the method of Hoffmann and Dedeken (1965). This method involves the colorimetric determination of the saligenin released when moistened soil (5 g) was incubated with 5 mL β-glucosido-saligenin (salicin) and 10 mL 2 M acetate buffer (pH 6.2) at 37°C for 3 h. Absorbance was measured at 578 nm and activity expressed in μg saligenin g^{-1} soil $(3\ h)^{-1}$.

Statistical Analysis

Results were calculated on basis of oven-dry soil weight (dw) and represent arithmetic Means±Standard Deviations (SD) of four lab replications for biological parameters. Statistical differences in enzyme activities between the cropping systems were determined (SAS, 1985) using a Randomized Complete Block Design (RCBD) and the mean separation of the Duncan Multiple Range Test (DMRT). Exploratory statistics in form of Principal Component Analyses (PCA) were conducted by SPSS 10.0 (SPSS, 2000). This multivariate analysis is based on the linear model of variance analysis. It consists of decomposing the total variability between enzyme activities, physico-chemical soil properties and cropping systems.

RESULTS

In general, as compared to irrigated Vertisols, rain-fed Vertisols (either cultivated or not) have higher clay content (average 63 Vs 56%), organic carbon (average 7.54 Vs 4.38 g kg^{-1} and total nitrogen (average 0.44 Vs 0.64 g kg^{-1}, Table 1). Activities of alkaline phosphatases were significantly (CV 20.5%, $p<0.001$) higher (605-747 μg p-nitrophenol g^{-1} soil h^{-1}) in the irrigated Rahad rotation and the three samples from the rainfed Vertisols (Rahd natural forest, sorghum-sesame and continuous sorghum) than (201-302 μg p-nitrophenol g^{-1} soil h^{-1}) for the two irrigated Vertisols (Gezira and Managil) (Fig. 1). In the rainfed Vertisols, cropping sequence or plant cover had no significant effect on the activities of alkaline phosphatases. However, within the irrigated Vertisols, by increasing duration of cultivation, enzyme activities decreased in the range of 12%, though not significant.

The activities of proteases (Fig. 2) revealed a similar pattern like alkaline phosphatases, with significantly (CV 44.7%, $p<0.04$) high activities [(12-15 μg tyrosine g^{-1} soil $(2\ h)^{-2}$] in the short-term cropping system of Rahad and the rain-fed systems of sorghum-sesame, continuous sorghum and the natural forest and low activities in Gezira, Managil and the Gezira permanent fallow [(12-15 μg tyrosine g^{-1} soil $(2\ h)^{-2}$]. The increasing duration of cultivation in the irrigated Vertisols has significantly reduced protease activities. Similar to alkaline phosphatase, within the rainfed Vertisols, cropping sequences had no effects on β-glucosidase activity. Activities of this soil enzyme were not statistically (CV = 24%, $p \leq 0.07$) affected by either cultivation period or cropping system (Fig. 3). However, highest activities were found in two samples (Natural grass Gezira and Rahad rotation) from the irrigated Vertisols of different cropping sequences. Both soils revealed a surprisingly different C_{org} (1.46% compared to 7.18%) and N_{tot} content (0.36% compared to 0.59%) and were not correlated (C_{org}: $r = 0.12$, $p<0.05$; N_{tot}: $r = 0.23$, $p<0.01$) with enzyme activity. The other soil samples showed lower activities with slight differences but without any statistical significance (CV 24.9%, $p<0.1$).

Properties that best determine enzyme activities varied significantly between each other (Table 4). Accordingly, enzyme activities of alkaline phosphatases were positively correlated with rainfall ($r = 0.98$, $p<0.001$), N_{tot} ($r = 0.95$, $p<0.001$), C_{org} ($r = 0.93$, $p<0.002$), clay and water supply. Whereas temperature ($r = -0.98$, $p<0.001$) and silt content ($r = -0.97$, $p<0.001$) were

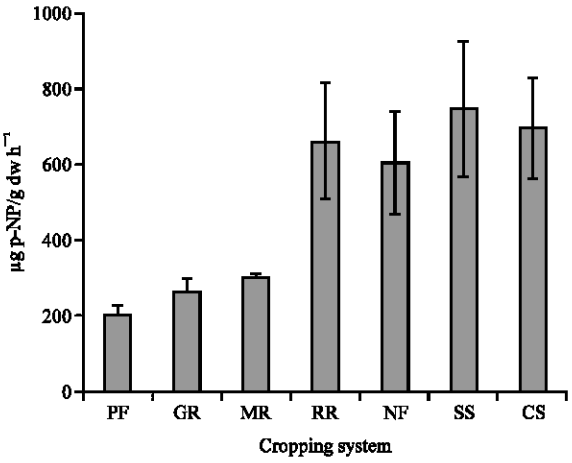

Fig. 1: Alkaline phosphatase activity of different cropping systems (vertical bars indicate±SD of the mean). $p<0.001$

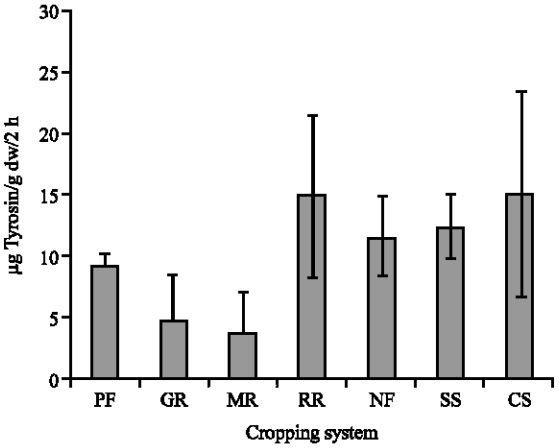

Fig. 2: Protease activity of different cropping systems (vertical bars indicate±SD of the mean). $p<0.04$

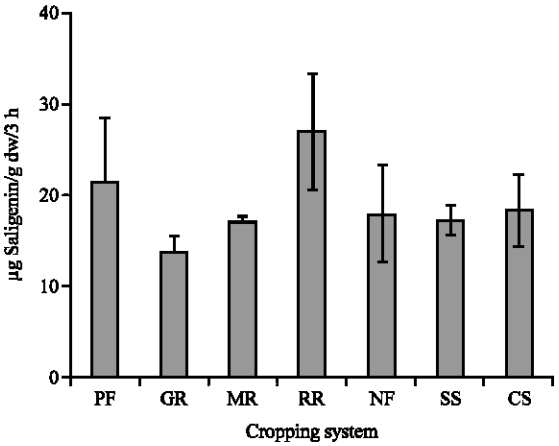

Fig. 3: β-glucosidase activity of different cropping systems (vertical bars indicate±SD of the mean). $p<0.07$

negatively correlated. The protease activities were positively correlated with rainfall and negatively with temperature and silt content. We found no correlations between β-glucosidase activities and physico-chemical soil properties.

Total N, organic C and clay content are significantly different among the three sites (Table 1). Rain fed areas contained higher total N, organic C and clay than irrigated areas. The seven soil treatments were clearly clustered by PCA into two groups (Fig. 4). The first group (consisting of Gezira natural grassland, Gezira rotation and Managil rotation) was characterized by low mean annual rainfall and high maximum/minimum temperatures, low N_{tot}- and C_{org}-content. In contrast, the soils of the second group (Rahad rotation, Rahad natural forest, Rahad sorghum-sesame and Rahad continuous sorghum) enzyme activities were influenced by higher mean annual rainfall and lower maximum/minimum temperatures, higher N_{tot}- and C_{org}-content.

The second PCA (Fig. 5) shows the important parameters that are responsible for the separation of sampling sites. Accordingly and with reference to the separation of

Table 4: Correlation coefficients and significances between physico-chemical soil properties and enzyme activities

	Enzyme		
Parameters	Alkaline phosphatase	Protease	β-Glucosidase
N_{tot}	0.945***	0.730	0.231
C_{org}	0.929***	0.646	0.121
C/N	0.675	0.311	-0.103
Sand	-0.028	-0.216	0.032
Silt	-0.969***	-0.844**	-0.260
Clay	0.843**	0.583	0.124
R_{mean}	0.975***	0.889**	0.348
W_{sup}	0.754**	0.568	-0.248
T_{max}	-0.975***	-0.889**	-0.348
T_{min}	-0.975***	-0.889**	-0.348

N_{tot}: Total N, C_{org}: Organic C, R_{mean}: Mean annual rainfall, W_{sup}: Water supply, T_{max}: Maximum annual temperature, T_{min}: Minimum annual temperature. *, **, ***indicate significant correlations at 5, 1 and 0.1%, respectively

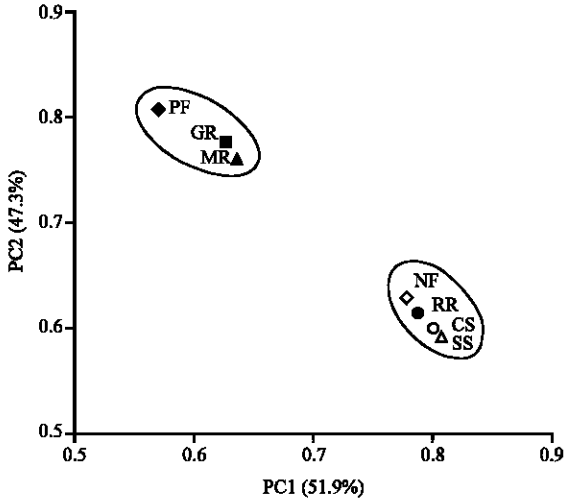

Fig. 4: Separation of investigated sampling sites by PCA

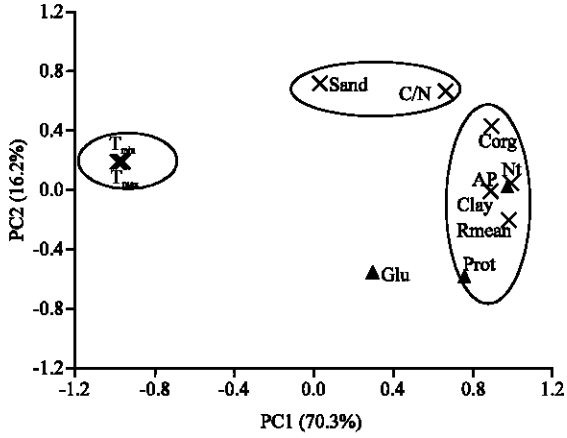

Fig. 5: Ordination plot of important parameters, responsible for the separation of sampling sites

investigated sites (Fig. 4), PC1, 51.9% of variances by the parameters silt and temperature of the left cluster and by alkaline phosphatase, protease and most of the abiotic parameters of the right cluster. Furthermore, for PC2, 47.3% of variances by the third cluster of sand and C/N ratio. The given parameters of the three clusters have the main influence on the differentiation of the sampling sites and treatments.

DISCUSSION

Higher total nitrogen and organic carbon in rainfed areas could possibly be due to better physical protection of organic matter and also higher retained plant residues. Increased levels of protection due to less tillage might possibly justify retention of higher clay content in the topsoil as downwards transport of fine soil particles are expected to be less in rainfed areas.

Soil enzyme activities have the potential to provide a unique integrative and reliable biological assessment of soils because of their relationship to soil biology, easy of measurement and rapid response to changes in soil management (Dick *et al*., 1994; Dick, 1997; Lulu and Insam, 2000). In former decades, enzyme activities have been used to monitor different issues of environmental quality. They have been tested as indicators of soil fertility (Waksman, 1922), soil quality (Castillo and Joergensen, 2001; Böhme *et al*., 2005), pollution impacts (Langer and Günther, 2001) and nutrient cycling (Visser and Parkinson, 1992). Tillage practices and different vegetation covers were found to influence enzyme activities (Bergstrom *et al*., 2000; Waldrop *et al*., 2000; Badiane *et al*., 2001). In temperate regions intensive agriculture depress soil enzyme activities, whereas cultivated soils in tropical regions which were amended with organic matter showed higher enzymatic activities than uncultivated soils (Dick *et al*., 1994). Especially, in water limited soil systems, the supply of plant available nutrients depends on enzyme activities (Klein *et al*., 1985). However, there is no agreement about the enzymes which are suitable best for different environmental assessments (Bruggen-Van and Semenov, 2000). Dalal (1982) reported that the amount of soil organic substances served as a carbon source that enhanced microbial biomass and consequently affected phosphatase activity, showing that this enzyme is of microbiological origin (Tarafdar and Classen, 1988; Chantigny *et al*., 2000; Klose and Tabatabai, 2002). Waldrop *et al*. (2000) obtained a higher enzyme activity in plantation soils than in forest soils and furthermore they found no correlation between enzyme activity and C_{org} or N_{tot}. Wick *et al*. (2002) described in their studies that there was no decline in enzyme activities during the dry season of a Nigerian soil and explained these findings by the protection of enzymes by Soil Organic Matter (SOM). They considered these enzymes as indicators for long-term changes of soil quality. Rojo *et al*. (2000) showed that phosphomonoesterases were poorly associated with stable, well-humified organic matter and rather better associated with fresh organic matter in the rough-textured soil fractions. It is well known that free extracellular enzymes (Skujins, 1976) could be adsorbed on the surface of soil particles (Hayano and Katami, 1977). Present findings indicate, a strong influence of silt on soil enzyme activities. Stemmer *et al*. (1998) studied organic matter and enzyme activity in particle-size fractions of soils. They reported highest invertase activities in silt and clay and were related to organic C. Kandeler *et al*. (1999) revealed a close relationship between enzyme activities and particle-size fractions, with xylanase and invertase being associated with coarse sand and the silt fraction. Matocha *et al*. (2004) obtained in different tillage treatments that the phenol oxidase was located primarily in the silt fraction, followed by clay and sand in no-tillage management.

We assume that our results of low phosphatase activities may indicate that long- and medium-term of intensive cropping systems of Gezira and Managil where many agro-chemicals (herbicides, fertilizers and pesticides) have been applied may indicate reduced biological activities. However, in the rainfed Vertisols, chemicals were never used indicating more favourable conditions for biota. In this respect, Bonmati *et al.* (1991) and Röver and Kaiser (1999) stressed that the variability of microbiological parameters is higher than those of chemical ones. Morris (1999) reported low variability of the chemical and physical soil properties and high variability of the microbial biomass over the area he sampled.

Present results of protease activity confirm the findings of Tate (1984), who showed that the addition of plant litter to soil increased the activity of proteases. The inducibility of proteases by farmyard manure application can be explained by the increased nitrogen demand to build up microbial biomass stimulated by organic substance amendment (Balakrishnan *et al.*, 2007). Kandeler *et al.* (1999) also related the increased activity of proteases to the intensified growth of the microbial community. Our findings of β-glucosidase activity are in accordance with the results found by Waldrop *et al.* (2000) and Badiane *et al.* (2001). They also obtained no relationship between β-glucosidases and C_{org} or N_{tot}. Other authors, however, described significant, positive correlation between β-glucosidases activity and C_{org} (Landgraf and Klose, 2002; Taylor *et al.*, 2002; Turner *et al.*, 2002) and considered this activity as a very sensitive biological indicator (Miller and Dick, 1995). This contradiction may be due to the fact that β-glucosidases can also occur as free extracellular enzymes (Skujins, 1976), adsorbed to clay minerals (according to Table 1, nearly 60%) and in humic acids-entrapped enzymes (Hayano and Katami, 1977; Burns, 1982). Positive impact of soil cover and lack of tillage on this soil enzymatic activities have already been shown by Gupta and Germida (1988) and Deng and Tabatabai (1996).

Present study showed, clear differences in the three enzyme activities as compared to previous studies. However, the observed differences in results could possibly be due to their origin and properties. The lower enzyme activities in the tropical region studied may result from differences in the type of clay minerals present in the soils from warmer regions. Clay mineral in cooler soils tend to be less weathered than those in soils from warmer regions, which tend to be highly weathered. This may be linked to lower expected microbial biomass, which was not measured in this study. In this respect, Alkaline phosphatases originate from microorganisms and animals, whereas proteases and β-glucosidases are produced by plants as well. Thus, uneven root and litter distribution cannot influence alkaline phosphatase activity in contrast to protease and β-glucosidase activities, which are impacted by these factors. Soil pH and phosphorus content, however, significantly affects alkaline phosphatase activity and controls phosphorus availability. Therefore, an altered pH value (maybe due to root exudates) and fertilizer amendment can influence alkaline phosphatase activity.

The most variable enzyme, protease can exist both extra- and intracellular, because it can be adsorbed to clays, embedded in the humic substances and located inside living microbial cells. Thus, the heterogeneity of soils caused by several biotic and abiotic properties might, at least in part, explain the high variability of protease activity (Böhme *et al.*, 2004).

The least variable enzyme, β-glucosidase is widely abundant, rarely substrate limited and it is synthesized by soil microorganisms in response to the presence of suitable substrate (Turner *et al.*, 2002). This enzyme has a strong relationship with the microbial biomass, which suggests that the activity of extracellular immobilized enzymes is negligible or even unimportant. For example, Kiss *et al.* (1972) stated that cellobiose degradation in soils was due to enzymes released from proliferating organisms rather than the accumulated enzyme fraction. Nowadays, however, several studies show a strong relationship between β-glucosidase activity and clay content, which may reflect the potential for enzyme

immobilization in the soil and therefore, the dominance of immobilized extracellular enzymes (Busto and Perez-Mateos, 2000; Turner *et al*., 2002). Thus, this enzyme is protected physically from degradation, stays active for a long time and is able to form an even distribution in soil. This might explain the reduced variability of β-glucosidase activity.

CONCLUSION

With exception of the β-glucosidase activities these results confirm that biological indicators are as important as chemical or physical parameters for soil assessment. The results provide information on three selected soil enzyme activities of the C-, N- and P-cycling influenced by duration of cultivation, sequences, water regimes (irrigated mechanized and traditional rainfed), soil and climatic conditions at seven field experiments. Soil enzymes were found to discriminate the treatments over decades, differed at the investigated sites and revealed microbiological relevant alterations of the soil ecosystems. Among the three different soil enzyme activities, alkaline phosphatase and protease appear to be the most sensitive enzymes for showing differences between irrigated and rainfed areas. Whereas β-glucosidase activities revealed no clear relationship neither with soil properties nor with management systems. Further research is needed to confirm this hypothesis and the role of enzyme activities in mineralization intensity and nutrient cycling in semi-arid tropical soils.

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REFERENCES

Badiane, N.N.Y., J.L. Chotte, E. Pate, D. Masse and C. Rouland, 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Applied Soil Ecol., 18: 229-238.

Balakrishnan, V., K. Venkatesan and K.C. Ravindran, 2007. The influence of halophytic compost, farmyard manure and phosphobacteria on soil microflora and enzyme activities. Plant Soil Environ., 53: 186-192.

Bending, G.D., M.K. Turner, F. Rayns, M.C. Marx and M. Wood, 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol. Biochm., 36: 1785-1792.

Bergstrom, A.W., C.M Monrea, A.D. Tomlin and J.J. Miller, 2000. Interpretation of soil enzyme activities in a comparison of tillage practices along a topograhic and textural gradient. Can. J. Soil Sci., 80: 71-79.

Böhme, L., F. Böhme and U. Langer, 2004. Spatial variability of enzyme activities in a 100-year old long-term field experiment. Biol. Fertil. Soils, 40: 153-156.

Böhme, L., U. Langer and F. Böhme, 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ., 109: 141-152.

Bonmati, M.C., B. Ceccanti and P. Nannipieri, 1991. Spatial variability of phosphatase, urease, protease, organic carbon and total nitrogen in soil. Soil Biol. Biochem., 23: 391-396.

Bourma, J., 2002. Land quality indicators of sustainable land management across scales. Agric. Ecosyst. Environ., 88: 129-136.

Bruggen-Van, A.H.C. and A.M. Semenov, 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecol., 15: 13-24.

Buraymah, I.M. and R. Webster, 1989. Variation in soil properties caused by irrigation and cultivation in the central Gezira of Sudan. Soil Till. Res., 13: 57-74.

Burns, R.G., 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol. Biochem., 14: 423-427.

Busto, M.D. and M. Perez-Mateos, 2000. Characterization of β-D-glucosidase extracted from soil fractions. Eur. J. Soil Sci., 51: 193-200.

Castillo, X. and R.G. Joergensen, 2001. Impact of ecological and conventional arable management systems on chemical and biological soil quality indices in Nicaragua. Soil Biol. Biochem., 33: 1591-1597.

Chantigny, M.H., D.A. Angers and C.J. Beauchamp, 2000. Active carbon pools and enzyme activities in soils amended with de-inking paper sludge. Can. J. Soil Sci., 80: 99-105.

Dalal, R.C., 1982. Effect of plant growth and addition of plant residues on the phosphatase activity in soil. Plant Soil, 66: 265-269.

Dawelbeit M.I. and E.A. Babiker, 1997. Effect of tillage and method of sowing on wheat yield in irrigated Vertisols of Rahad, Sudan. Soil Till. Res., 42: 127-132.

Deng, S.P. and M.A. Tabatabai, 1996. Effect of tillage and residue management on enzyme activities in soils: II. Glycosidases. Biol. Fertil. Soils, 22: 208-213.

Dick, W.A., 1984. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci. Soc. Am. J., 48: 569-574.

Dick, R.P., P.E. Rasmussen and E.A. Kerle, 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils, 6: 159-164.

Dick, R.P., 1994. Soil Enzyme Activities as Indicators of Soil Quality. In: Soil Enzymes, Doran, J.W., D.C. Coleman, D.F. Bezdicek and B.A. Stewart (Eds.). Soil Science Society of America, Madison, WI, pp: 107-124.

Dick, R.P., J.A. Sandor and N.S. Eash, 1994. Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agric. Ecosyst. Environ., 50: 123-131.

Dick, R.P., 1997. Soil Enzyme Activities as Integrative Indicators of Soil Health. In: Biological Indicators of Soil Health, Pankhurst, C.E., B.M. Doube and V.V.S.R. Gupta (Eds.). CAB International, Wallingford, UK., pp: 121-156.

Doran, J.W. and M.R. Zeiss, 2000. Soil health and sustainability: Managing the biotic component of soil quality. Applied Soil Ecol., 15: 3-11.

El-Awad, S.E.D.A.G., 2000. Effects of irrigation interval and tillage systems on irrigated cotton and succeeding crop under heavy clay soil in the Sudan. Soil Till. Res., 55: 167-173.

Filip, Z., 2002. International approach to assessing soil quality by ecological-related biological parameters. Agric. Ecosyst. Environ., 88: 169-174.

Freeland, P.W., 1977. Characterization of soil samples by enzyme activities. J. Biol. Educ., 11: 27-32.

Gupta, V.V.S.R. and J.J. Germida, 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem., 20: 777-786.

Hayano, K. and A. Katami, 1977. Extraction of β-glucosidases activity in soil. Soil Biol. Biochem., 9: 349-351.

Herrick, J.E., 2000. Soil quality: An indicator of sustainable land management? Applied Soil Ecol., 15: 75-83.

Hoffmann, G. and M. Dedeken, 1965. Eine methode zur colorimetrischen Bestimmung der β-Glucosidase-Aktivität im Boden. Z. Pflanzenernähr. Düng. Bodenk. 108: 193-198.

Hurni, H., 2000. Assessing sustainable land management (SLM). Agric. Ecosyst. Environ., 81: 83-92.

Kandeler, E., J. Luxhøi, D. Tscherko and J. Magid, 1999. Xylanase, invertase and protease at the soil-litter interface of a loamy sand. Soil Biol. Biochem., 31: 1171-1179.

Kiss, S., M. Dragan-Bularda and F.H. Khaziev, 1972. Influence of chloromycetin on the activities of some oligases of soils. Proceedings of the Lucrări Conferintã Nationalã Stiintã Solului, 1972, Iasi, pp: 451-462.

Klein, D.A., D.L. Sörensen and E.F. Redente, 1985. Soil Enzymes: A Predictor of reclamation Potential and Progress. In: Soil Reclamation Processes: Microbiological Analyses and Application, Tate, R.L. and D.A. Klein (Eds.). Marcel Dekker, New York, ISBN: 0824772865, pp: 141-171.

Klose, S. and M.A. Tabatabai, 2002. Response of phosphomonoesterases in soils to chloroform fumigation. J. Plant Nutr. Soil Sci., 165: 429-434.

Körschens, M. and A. Weigel, 1998. The Evidence of Carbon Dynamics in Soil, Investigation in Pot and Model Experiments. In: Resource Management in Fragile Environments, Behl, R.K., A.P. Gupta, A.L. Khurana and A. Singh (Eds.). CCS HAU, Hisar and MMB, New Delhi, ISBN: 88-464-5357-3, pp: 123-132.

Ladd, J.N. and J.H.A. Butler, 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem., 4: 19-30.

Landgraf, D. and S. Klose, 2002. Mobile and readily available C and N fractions and their relationship to microbial biomass and selected enzyme activities in a sandy soil under different management systems. J. Plant Nutr. Soil Sci., 165: 9-16.

Langer, U. and T. Günther, 2001. Effects of alkaline dust deposits of phosphate fertilizer production on microbial biomass and enzyme activities in grassland soils. Environ. Pollut., 112: 321-327.

Loeppert, R.H. and D.L. Suarez, 1996. Carbonate and Gypsum. In: Methods of Soil Analysis, Part 3 Chemical Methods, Sparks, D.L. (Ed.). Soil Sci. Soc. Am., Madison, WI., ISBN: 1-56238-407-4.

Lulu, B. and H. Insam, 2000. Medium-term effects of a single application of mustard residues on soil microbiota and C content of vertisols. Biol. Fertil Soils, 31: 108-113.

Matocha, C.J., G.R. Haszler and J.H. Grove, 2004. Nitrogen fertilization suppresses soil phenol oxidase enzyme activity in no-tillage systems. Soil Sci., 169: 708-714.

Mclean, E.O., 1982. Soil pH and Lime Requirement. In: Methods of Soil Analysis, Part 2, Page, A.L. (Ed.). ASA and SSSA, Madison, WI., ISBN: 0-8493-0022-3, pp: 192-224.

Miller, M. and R. Dick, 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biol. Biochem., 27: 1161-1166.

Morris, S.J., 1999. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: Fine scale variability and microscale patterns. Soil Biol. Biochem., 31: 1375-1386.

Mubarak, A.R., O.M.E. Elshami and A.A. Azhari, 2005. Long and short-term effects of conventional tillage on a vertisol properties under Sugarcane plantation. Soil Till. Res., 84: 1-6.

Pascual, J.A., C. Garcia, T. Hernandez, J.L. Moreno and M. Ros, 2000. Soil microbial activity as a biomarker of degradation and remediation processes. Soil Biol. Biochem., 32: 1877-1883.

Rojo, R.R., M.G.D. Mendoza, B.C.M. García, G.J.R. Bárcena and I.E. Aranda, 2000. Intake and diegestibility of tropical grasses in steers with nitrogen supplementation and *Saccharomyces cereisiae*. Rev. Fac. Agron. (LUZ.), 17: 358-370.

Röver, M. and E.A. Kaiser, 1999. Spatial heterogeneity within the plough layer: Low and moderate variability of soil properties. Soil Biol. Biochem., 31: 175-187.

Salih, A.A., H.M. Babiker and S.A.M. Ali, 1998. Preliminary observations on effects of tillage on soil physical properties, cotton growth and yield in Gezira Scheme, Sudan. Soil Till. Res., 46: 187-191.

SAS, 1985. SAS User's Guide: Statistics. 5th Edn., SAS Institute, Cary, NC. USA., ISBN: 091738265X.

Schulz, E., 2004. Influence of site conditions and management on different soil organic matter (SOM) pools. Archiv. Agron. Soil Sci., 50: 33-48.

Sherwood, S. and N. Uphoff, 2000. Soil health: Research, practice and policy for a more regenerative agriculture. Applied Soil Ecol., 15: 85-97.

Skujins, J., 1976. Extracellular enzymes in soil. Crit. Rev. Microbiol., 4: 383-421.

Soil Survey Staff, 1996. Keys to Soil Taxonomy. 7th Edn., United States Department of Agriculture, Washington, D.C. ISBN-13: 978-0932865106.

SPSS, 2000. SPSS 10.0 for Windows. SPSS Inc., Chicago, USA., ISBN-10: 0130284262.

Stemmer, M., M.H. Gerzabek and E. Kandeler, 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biol. Biochem., 30: 9-17.

Tabatabai, M.A. and J.M. Bremner, 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem., 1: 301-307.

Tabatabai, M.A., 1994. Enzymes. In: Methods of Soil Analysis Microbial and Biochemical Properties Part 2, Weaver, R.W., S. Augle, P.J. Bottomly, D. Bezdicek, S. Smith, A. Tabatabai and A. Wollum (Eds.). SSSA, Madison, WI., ISBN: 089118810X, pp: 775-833.

Tarafdar, J.C. and N. Claassen, 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soils, 5: 308-312.

Tate, R.L., 1984. Function of protease and phosphatase activites in subsidence of Pahokee muck. Soil Sci., 138: 271-278.

Taylor, J.P., B. Wilson, M.S. Mills and R.G. Burns, 2002. Comparison of microbial numbers and enzymatic activities in surface soils and sub soils using various techniques. Soil Biol. Biochem., 34: 387-401.

Turner, B.L., D.W. Hopkins, P.M. Haygarth and N. Ostle, 2002. β-Glucosidase activity in pasture soils. Applied Soil Ecol., 20: 157-162.

Visser, S. and D. Parkinson, 1992. Soil biological criteria as indicators of soil quality: Soil microorganisms. Am. J. Alt. Agric., 7: 33-37.

Von-Wirén-Lehr, S., 2001. Sustainability in agriculture-an evaluation of principal goal-oriented concept to close the gap between theory and practice. Agric. Ecosyst. Environ., 84: 115-129.

Waksman, S.A., 1922. Microbiological analysis of soil as an index of soil fertility. III. Influence of fertilization upon numbers of microorganisms in the soil. Soil Sci., 14: 321-346.

Waldrop, M.P., T.C. Balser and M.K. Firestone, 2000. Linking microbial community composition to function in a tropical soil. Soil Biol. Biochem., 32: 1837-1846.

Wick, B., R. Kühne, K. Vielhauer and P. Vlek, 2002. Temporal variability of selected soil microbiological and biochemical indicators under different soil quality conditions in South-western Nigeria. Biol. Fertil. Soils, 35: 155-167.