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By
Hamid Kheyrodin and Khosro Ghazvinian

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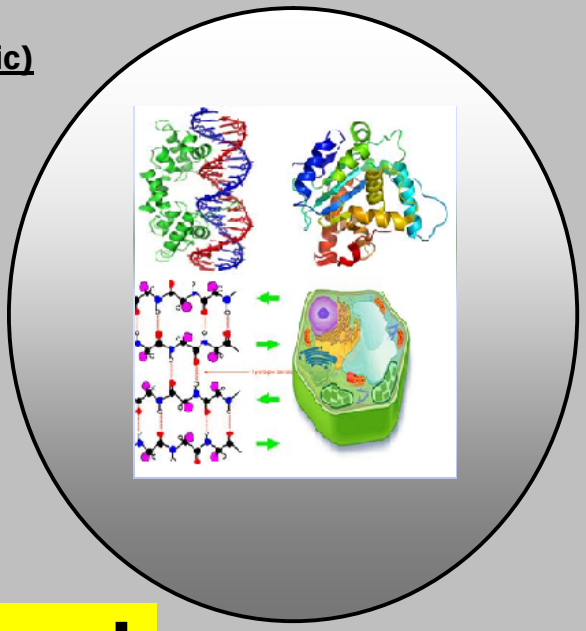

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Soil Enzyme Activities and Microbial Biomass Activities in Farmland Soil

***Hamid Kheyrodin and **Khosro Ghazvinian**

***Faculty of Desert Science-Semnan University, Iran.**
****Faculty of Veterinary and Animal Science-Semnan University, Iran.**

ABSTRACT

Soil enzymes activities and microbial biomass have an important influence on nutrient cycling. The spatial distribution of microbial biomass and soil hydrolysis activities involved in soil C-, N-, and P-cycle was examined in the farmlands of Iran. Significant (P < 0.05) correlations were observed between latitude and activities of invertase and acid phosphatase, and the levels of MBC and MBN and the MBC/MBN ratio, which increased with the latitude. The spatial distribution of soil enzyme activities and microbial biomass depended on the contents of soil organic matter along the latitudinal gradient. The higher microbial biomass and activities of invertase and acid phosphatase in the north than in the south indicated the faster turnover rate of soil C-, N-, and P-cycle, which might accelerate soil organic matter decomposition and improve crop growth. Additionally, soil pH and EC had some impacts on soil enzyme activities. Significant positive correlations (P <0.05) were observed between invertase activity and the total N and available P, and between acid phosphatase activity and available N, total P. The urease, acid phosphatase, and dehydrogenase activities were significantly correlated with the soil pH and electrical conductivity (EC). MBC and MBN were positively correlated with the total C, C/N, and available P. The MBC/MBN ratio was correlated with the total C, total N, C/N, and available N. The distribution of soil enzyme activities and microbial biomass resulted from the changes in soil properties such as soil organic matter, soil pH, and EC, partially owing to variations in temperature.

Key words: Soil, Enzymes activities, Microbial biomass, Soil chemical properties, Nutrients cycles.

INTRODUCTION

Soil management, which uses traditional plowing and disking to prepare the land, may reduce soil organic matter (SOM) and microbial activity (Dick, 1984, Doran, 1980 and Gupta, and Germida, 1988). Consequently, agricultural practices toward smaller soil degradation are needed to improve soil quality and agricultural sustainability. No-tillage, planting with minimal soil disturbance combined with crop rotation protects the soil against degradation toward sustainability. Tillage alters soil structure exposing more organic matter to microbial attack while no-tillage practices stimulate the formation and stabilization of macro aggregates, which represent an important mechanism for protection and maintenance of SOM (Beare, et al. 1994) besides other effects as more stable temperature and changes in the distribution of organic matter and nutrients in the soil (Dick, 1984). The SOM decomposition is mediated by microorganisms, which have their activity stimulated on tropical soils where temperature is higher than temperate climate (Coleman, et al. 1989). Crop rotations that have diverse crop sequences also can be important for maintaining and improve soil quality. Crop rotations change the soil habitat due to their difference in extract nutrients, depth of roots, amount of residue, which remain in soil and difference in their components. Crop rotations can stimulate soil biodiversity and biological activity over mono culturing. Soil management as no-tillage and crop rotations are important practices, which can reduce soil erosion, conserve organic matter and water and stimulate microbial activity (Dick, 1984, Doran, 1980 and Gupta, and Germida, 1988). 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, et al. 1994). Although enzymes are primarily of microbial origin it can also be 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 (Dick, 1997 and Tabatabai, 1994). Soil enzyme activities have successfully discriminated between wide ranges of soil management practices (Dick, 1997 and Gupta, and Germida, 1988). It is known as well, that the excessive cultivation can cause decrease in the microbial biomass and its activity (Gupta, and Germida, 1988). Dick (1994) showed that activities of some enzymes were higher in NT than CT in top 7.5 cm layer. Although there is a lot of information that show the relation between soil management and soil enzymes activities, very little is known about these effects under tropical/subtropical conditions (Deng, and Tabatabai, 1996**,** Dick, et al, 1988 and Dick, 1994). In this context, the measurement of soil enzymes can be used as indicative of the biological activity or biochemical process. Soil enzyme activities have potential to provide a unique integrative biological assessment of soils because of their relationship to soil biology, easy of measurement, and rapid response to changes in soil management (Bandick, and Dick, 1999**,** Dick, 1994**,** and Dick, 1997).

The objectives of this study were determining the soil enzyme activities in soil under long-term crop rotations and tillage systems. We hypothesized that no-tillage and/or different crop rotation used would stimulate the enzyme activity.

MATERIAL AND METHODS

Experimental site and soil samples

A study of crop rotations and tillage established in 1976 was conducted at the Experimental Station of Agronomic Institute of Paraná (IAPAR), district of Londrina, State of Paraná, in the south region of Brazil (23º40' S, 50° 52' W and 576 m altitude). The soil is classified as Oxisol (Typic Haplorthox) with 85% of clay, 12% of silt, 3% of sand, pH 4.6 and content of organic C and P according Table 1. The experiment design was a split plot where tillage systems were the main plots (65 x 25 m) and crop rotations (soybean/wheat, S/W; maize/wheat, M/W and cotton/wheat, C/W) were the subplots (8 x 25 m separated by 2.0 m) with three replicates. No-tillage (NT) consists of planting crops in untilled soils by opening a narrow slot deep enough to cover the seed and conventional tillage (CT) consists of one deep discing and two light discings with a harrow for leveling the ground and preparing the seedbed. The fertilizers have been added according to the soil analysis done before each cropping. N fertilizer was never applied to the soybean crop. Five soil sub-samples were taken randomly from each subplot at 0-5, 5-10 and 10-20 cm depths in August 1997 and 1998 (at the end of the winter crop). The samples were composted, homogenized and sieved through a 4 mm screen after removing any large plant material.

Table 1. Total C and extractable P in soils under different tillage and crop rotations systems.

Crop Rotation[3]	Total C [1]		Extractable P[2]	
	CT	NT	CT	NT
	gkg^{-1}		$mgkg^{-1}$	
	0-5cm			
S/W	15.3	20.6	18.2	79.1
M/W	14.7	22.4	15.5	73.6
C/W	13.9	20.6	21.7	122.8
	5-10cm			
S/W	13.4	17.3	17.9	27.0
M/W	15.3	19.0	12.8	29.0
C/W	13.2	19.7	20.4	44.3
	10-20cm			
S/W	14.4	16.3	19.1	10.8
M/W	15.6	17.2	18.0	9.7
C/W	13.8	16.2	12.3	14.9

[1]Carbon determination by Walkley and black, [2]Extractable P by Mehlich, [3]S. Soybean, W: Wheat, M: Maize and C: Cotton.

Enzyme activities analysis

Amylase (EC 3.2.1) and cellulase (EC 3.2.1.4) were determined according modified methodology of Pancholy and Rice (1973) and Deng and Tabatabai (1994a). Arylsulfatase (arylsulfate sulfohydrolase, (EC 3.1.6.1) was determined by method of Tabatabai (1994) and acid and alkaline phosphatase (EC 3.1.3) was determined by the method of Tabatabai (1994) with a modified universal buffer (MUB) (pH 6.5 for acid phosphatase or pH 11.0 for the alkaline phosphatase).

All determinations were made in triplicate and expressed on a dry weight basis. For amylase and cellulase activity results are expresses µg glucose (GLU) g^{-1} d^{-1}. For activities of arylsulfatse and phosphatase results are expressed as µg *p*-nitrophenol (PNP) g^{-1} h^{-1}. Data were analyzed using the SAS statistical package (SAS Inst., 1998).

RESULTS AND DISSCUSSION

Amylase activity

Amylase activity varied from 350 to 730 µg g^{-1} d^{-1} in the CT plots and from 573 to 829 µg g^{-1} d^{-1} in the NT plots. NT resulted in a significant increase in amylase activity in all crops and depths with exception in maize at 5-10 cm depth where CT resulted in values 23% higher than NT. NT increased amylase activity over CT from 45 to 69% in 0-5 cm depth, 65 to 76% in 5-10 cm depth and from 8 to 28% in 10-20 cm layer. Crop rotation influenced amylase activity at 5-10 and 10-20 cm depth under CT systems, and the M/W rotation was 20% higher than S/W, and 11% higher than C/W rotation. In general amylase activity under NT was 37% higher than CT systems.

Cellulase activity

Cellulase activity varied from 67 to 139 mg g^{-1} d^{-1} in the CT plots and from 90 to 220 µg g^{-1} d^{-1} in the NT plots. NT resulted in a significantly increase in cellulase activity only at the first layer where maize and cotton showed higher cellulase activity than CT systems. Crop rotation influenced cellulase activity only at 5-10 cm under CT systems, where M/W rotation showed higher activity than other rotations. In general cellulase activity under NT was 37% higher than CT systems while the M/W rotation presented amylase activity 9% and 11% higher, respectively, than S/W and C/W.

Arylsulfatase activity

Arylsulfatase activity varied from 4.11 to 12.52 µg g^{-1} h^{-1} in the CT plots and from 19.17 to 32.65 µg g^{-1} h^{-1} in the NT plots. NT manage resulted in a significantly increase in arylsulfatase activity in all crop rotations at all depths. The increases over CT observed were from 110 to 288% at 0-5 cm depth, from 116 to 539% at 5-10 cm depth and from 240 to 353% at 10-20 cm depth. Crop rotation influenced arylsulfatase activity at 5-10 cm depth under CT while under NT systems there were effects of crop rotation at 0-5 cm and 10-20 cm depth. All of these effects were observed in M/W rotation.

In general arylsulfatase activity under NT was 215% higher than CT systems while the M/W rotation had 37% and 27% higher arylsulfatase activity than S/W and C/W, respectively.

Acid phosphatase activity

Acid phosphatase activity varied from 458 to 625 μg g^{-1} h^{-1} in the CT plots and from 633 to 852 μg g^{-1} h^{-1} in the NT plots. NT resulted in a significant increase in acid phosphatase activity in all crop rotations with exception in maize rotation at 5-10 cm and soybean at 10-20 cm depth. The increases in acid phosphatase activity due to soil management were from 28 to 68% at 0-5 cm depth, from 31 to 46% at 5-10 cm depth and from 12 to 50% at 10-20 cm depth. Crop rotation under CT influenced acid phosphatase activity at all depths, where C/W had lower activity than other crop rotations. In general acid phosphatase activity under NT was 8% higher than CT systems while M/W had 6% and 10% higher activity than S/W and C/W, respectively.

Alkaline phosphatase activity

Alkaline phosphatase activity varied from 75 to 147 μg g^{-1} h^{-1} in the CT plots and from 139 to 207 μg g^{-1} h^{-1} in the NT plots. NT resulted in a significantly increase in alkaline phosphatase activity in all crops at 0-5 cm, in S/W and C/W rotations at 5-10 cm and in M/W at 10-20 cm depth. As observed for acid phosphatase the alkaline phosphatase activity also was influenced by crop rotation under CT at all depths. Alkaline phosphatase activity was lower in C/W rotation than other crops, as observed in acid phosphatase. In general alkaline phosphatase activity under NT was 47% higher than CT systems while maize presented activity 10% and 28% higher, respectively, than soybean and cotton.

DISCUSSION

Amylase activities observed were similar to those (from 50 to 840 μg g^{-1}) obtained under different vegetation types by Pancholy and Rice (1973) while the cellulase activity were also similar those 95 μg g^{-1} under CT and 133 μg g^{-1} under NT (Deng, and Tabatabai, 1996). This trend of higher amylase activity under NT than CT is related to total soil C content. Tillage only affected cellulase activity in the surface depth when NT had 90% greater activity than CT which corresponded to 45% greater total C. Amylase and cellulase did not decreased with soil depth and a decrease in organic C as observed previously by Deng and Tabatabai (1996). These enzymes have an important role in residue decomposition. For example, cellulose is the most abundant compound in the biosphere, comprising almost half of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson, et al. 1990). So it is important to understand the factors that affect the degradation of cellulose in soils because the reactions involved provide readily available C for the growth of microorganisms (Deng, and Tabatabai, 1994b).

Greater arylsulfatase activity (234%) obtained in 0-20 cm under NT confirms a previous investigation that mulching and NT increase arylsulfatase activity significantly (Deng, and Tabatabai, 1997).

However we did not observe decreased of arylsulfatase activity with increase of soil depth as observed previously by Deng and Tabatabai (1997). Arylsulfatase is the enzyme that is involved in mineralization of ester sulfate in soils (Tabatabai, 1994), and its activity has varied widely in the literature in relation to soil properties and management (Bandick, and Dick, 1999**,** Dick et. al. 1988**,** Frankenberger, and Dick, 1983 and Gupta, and Germida, 1988). The greater arylsulfatase activity under NT may reflect the increase of fungal biomass because arylsulfatase has strong correlation with ergosterol (Taylor and Dick, unpublished), which is almost exclusively found in fungi (Newell, et al. 1987). Furthermore, fungi have up to 42% of its S as ester sulfate, which is the substrate for arylsufatase, while bacteria have around 10% ester sulfate-S (Saggar, et al. 1981). This is consistent with Frey *et al.* (1999) who found greater fungal than bacteria under reduced tillage. They observed that fungal hyphal length was 1.9 to 2.5 times higher in NT than CT. One of the reasons is that NT facilitates establishment and maintenance of hyphal compared to tillage that disrupts fungal networks. The 46% increase of acid phosphatase and 61% increase of alkaline phosphatase activities due to NT in the surface layer show these enzymes are sensitive to disturbance. Phosphatases are a broad group of enzymes that hydrolyzes esters and anhydrides of phosphoric acid. Both acid and alkaline phosphatase activity varies widely due to soil management (Dick, 1994 and Gupta, and Germida, 1988), fertilizer (Gupta, and Germida, 1988), and tillage (Kandeler, et al. 1999). Our observation of higher soil enzyme activities under NT than CT is in agreement with other studies. For example Deng and Tabatabai (1997) who observed higher values of arylsulfatase (30%), acid phosphatase (17%) and alkaline phosphatase (40%) under NT than CT systems. However, unlike most other studies of NT where enzyme activities decrease below the top 5 cm our study showed activities remaining fairly constant down to 20 cm. Most of the previous studies were in temperate regions, which have cold winter. Our study was in subtropical setting where soils remain warm year-around, which facilitates high rates of decomposition, reducing the potential to build up organic matter at the surface. This is reflected in total C levels which were only slightly higher in the 0-5 cm depth them lower depths in our study.

A fairly consistent effect of crop rotation was that M/W had significantly higher enzyme activities under CT compared to other crop rotations. This effect is likely due to the high biomass production of maize (about 9 tons ha^{-1} yr^{-1}). This would produce greater amounts of substrate for microbial growth and production of enzymes. Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues entering the soil, and its spatial distribution. While in CT systems, organic matter is more thoroughly distributed than in NT systems where crop residues are concentrated on the soil surface. Mulching, generally, increases enzymes activities in soils. With the increasing of mulch there is an increased of the supply of the readily available substrate, such as carbohydrates, for microorganisms as well as soil enzymes.

As a consequence, can occurs an increase in glycosidase activities because these enzymes play a major role in degradation of carbohydrates in soils and the hydrolysis of these enzymes are believed to be important energy sources for the growth of soil microorganisms (Deng, and Tabatabai, 1996). Glycosidases are likely involved in C cycling by catalyzing decomposition and releasing energy source such as glucose (Deng, and Tabatabai, 1996). Our results on a subtropical soil that NT increases the microbial biomass and enzyme activities are consistent with previous studies in temperate regions (Deng, and Tabatabai, 1996, Deng, and Tabatabai, 1997**,** and Kandeler, Tscherko, and Spiegel, 1999). The high concentration of residue and roots of previous crops in the surface soil under NT can affect its microbial activity. One of those benefits effects due to NT may be by "rhizosphere effect", which probably contributes significant for higher enzyme activities when compared with CT systems (Bandick, and Dick, 1999). Rhizosphere is a zone where there is an increase in microbial and enzyme activity (Bopaiah, and Shetty, 1991). Some enzyme activities (amylase, cellulase and invertase) can be more influenced by type of organic matter than the quantity of organic matter (Pancholy, and Rice, 1973), once had been observed that mineralization of plant residue added to soil is controlled by C:N ratio, and lignina, polyphenol and silica content (Pancholy, and Rice, 1973). These crop rotation effects can be also due to different exudate and organic components from root systems and crop residues, which influence microbial activity differently. In general has been accepted that there are a decrease in microbial activity with the increase of cultivation. As observed by Gupta and Germida (Gupta, and Germida, 1988) who found lower enzyme activities in all aggregates size fractions under cultivated soils than native soils. As enzymes play an important role in the biochemical mineralization of nutrients, these decreases in enzyme activities under CT might explain the lower microbial biomass and activities observed in the same experimental site (Balota, et al. 2004**,** Balota, et al. 2003**,** and Balota, et al. 1998).

Simple correlation across all treatments and depths (Table 2) showed that soil enzyme activities were significantly correlated with C microbial biomass. This indicates that enzyme activities were associated with active microorganisms in soil which are the major source of soil enzymes. While the significant correlation between enzyme activities and organic C is likely due to higher C levels supporting greater microbial biomass that is more activity. Furthermore, higher organic matter provides a better environmental for stabilizing and protecting extra cellular enzymes. The activities of all five enzymes were significantly inter correlated which suggest that tillage and crop rotations systems have similar effects on the activities of those enzymes involved in C, N, P and S cycling in soils (Deng, and Tabatabai, 1997).

Table 2. Simple correlation between soil enzyme activities, microbial and chemical properties across all treatments.

Variable	Amylase	Cellulase	Arylsulfatase	Acid phosphatase	Alkaline phosphatase
Amylase	-	-	-	-	-
Cellulase	0.68*	-	-	-	-
Arylsulfatase	0.60*	0.47	-	-	-
Acid phos	0.68*	0.64*	0.73*	-	-
Alkaline phose	0.64*	0.56*	0.72*	0.89*	-
Microbial properties					
MBC f.	0.75*	0.86*	0.65*	0.83*	0.74*
MBC ext.	0.59*	0.86*	0.43	0.79*	0.67*
MBN	0.50	0.44	0.24	0.55	0.57
MBP	0.28	0.52	0.07	0.31	0.15
MBS	0.01	0.001	0.02	0.04	0.04
C Mineralize.	0.04	0.015	0.05	0.10	0.11
N Mineralize	0.08	0.02	0.11	0.19	0.17
S Mineralize	0.01	0.001	0.01	0.02	0.02
Basal Respiration	0.33	0.34	0.16	0.20	0.32
qCO2	-0.37	-0.37	-0.35	-0.51	-0.35
Cmin:Corg	0.63*	0.70*	0.80	0.64*	0.55
Chemical properties					
C organic	0.67*	0.66*	0.80*	0.81*	0.79*
PH	0.26	0.19	0.45	0.49	0.49
P	0.46	0.64	0.31	0.47	0.34
Ca	0.28	0.14	0.35	0.43	0.31
Mg	0.34	0.23	0.51	0.60	0.55
CEC	0.51	0.56	0.56	0.56	0.45
Base saturation	0.13	0.03	0.19	0.29	0.23

MBCf: Microbial biomass carbon by fumigation- incubation method; MBC ext: Microbial biomass carbon by extraction method; MBN: Microbial biomass nitrogen; MBP: Microbial biomass phosphorus; MBS: Microbial biomass suphur. PH: Cacl2 0.01M. P: Extractable (Mehlich). * Significantly at $p < 0.05$.

We found no significant correlation between enzyme activities and soil pH. Similar observations also have been found (Baligar**,** et al. 1999, Eriksson**,** et al. 1990 and Fernandes, et al. 1998) even though phosphatases have been often closely correlated with soil pH (Deng, and Tabatabai, 1997, Dick, et. al, 1998, Fernandes, et al. 1998., and Juma, and Tabatabai, 1978). This because acid phosphatase predominanes in acid soils and alkaline phosphatase predominanes in alkaline soils (Juma, and Tabatabai, 1978). However, in our study this lack of correlation with pH may be due to the narrow range at pHs 4.1 and 4.7.

There was no correlation between acid and alkaline phosphatase activities with extractable P, which is consistent with other studies (Baligar, et al. 1999 and Eriksson, et al. 1990). This lack of correlation between phosphatases and extractable P may be due to the suppression of soil phosphatase activity from long-term application of phosphate fertilizer (about 125 kg P_2O_5 per ha per year) as suggested by Haynes and Williams (Haynes, and Williams, 1992). Thus, it seems phosphatases are stimulated when phosphate levels are low in soils (Spiers, and McGill, 1978).

Although the ecological significance of specific soil enzymes activities is still debatable (Nannipieri, et al. 1990) there are several works which show, in a clear way, the effects of soil management in enzyme activities (5,11,12,14,16,24,27). The increase in soil enzyme activities may be the result of soil physical and chemical changes, so there is a direct expression on microbial biomass and soil enzyme activities. One argument, which can explain the increase in soil enzyme activities due to tillage, is that NT can improve the microbial habitat. Long-term tillage alters soil structure and can increase the losses of organic matter, because of tillage disrupt soil aggregates exposing more organic matter to microbial attack (Beare, et al. 1994). The formation and stabilization of macroaggregates under NT soil represent an important mechanism for the protection and maintenance of soil organic matter that is lost under CT practices (Beare, et al. 1994). Thus, macroaggregates provide an important microhabitat for microbial activity (Dick, R.P. 1992). Higher organic matter levels support greater microbial activity because of greater supplies of energy and nutrients. Additionally, greater humic content could facilities incorporation of soils enzymes into the soil matrix allowing stabilization of higher exoenzymes in soils because humic compounds are important in soil enzyme complexation (Dick, 1994 and Paul, and McLaren, 1975).

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Corresponding author: Dr. Hamid Kheyrodin, Faculty of Desert Science-Semnan University, Iran**. Email:** hkhyrodin@yahoo.com