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Effect of Different Fertilization Methods on soil Biological Indexes

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Abstract**—**Fertilization plays an important role in crop growth and soil improvement. This study was conducted to determine the best fertilization system for wheat production. Experiments were arranged in a complete block design with three replications in two years. Main plots consisted of six methods of fertilization including (N1): farmyard manure; (N2): compost; (N3): chemical fertilizers; (N4): farmyard manure + compost; (N5): farmyard manure + compost + chemical fertilizers and (N6): control were arranged in sub plots. The addition of compost or farm yard manure significantly increased the soil microbial biomass carbon in comparison to the chemical fertilizer. The dehydrogenase, phosphatase and urease activities in the N3 treatment were significantly lower than in the farm yard manure and compost treatments.

Keywords**—**Enzyme activity, Fertilization, Microbial biomass carbon, Wheat

I. Introduction

CONVENTIONAL wheat production utilizing tillage, commercial fertilizer applied through pesticides, and irrigation can improve the grain yield. However, this intensive production system also can degrade soil quality, enhance runoff by covering the soil with an impervious surface, contribute to surface and impurity pollution and add to production cost [25]. Alternative systems have been developed that use renewable organic resources and minimize tillage to build soil organic matter and enhance soil quality. Fertilization is one of the soil and crop management practices, which exert a great influence on soil quality [3]. Farmyard manure and compost are organic sources of nutrients that also have been shown to increase soil organic matter and enhance soil quality. It is well known that organic amendments, such as plant residues, farm yard manures and composts have a number of benefits in soil physical and chemical properties. Many reports have also revealed different aspects of biology of soils amended with organic matters, including the number of general microorganisms [23], biomass of bacteria and fungi [14], enzyme activities [10] and biochemical properties [15]. Microbial communities perform necessary ecosystem services, pathogen suppression, stabilization of soil aggregates, and degradation of xenobiotics.

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Soil microbial biomass, activity, and community structure have been shown to respond to agricultural management practices. Alternation to no tillage or increased cropping intensity increases microbial biomass C (MBC) in response to increase nutrient reserves and improved soil structure and water retention [2].

Enzyme activities have been indicated as soil properties suitable for use in the evaluation of the degree of alteration of soils in both natural and agroecosystems. Soil microbial properties have a strong correlation with soil health. Some research has already suggested the favorable effects of conservation tillage practices and organic fertilizers on soil enzyme activities [10]. The activity of dehydrogenase is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity, because it is exclusively intracellular and, theoretically, can function only within viable cells. Urease catalyses the hydrolysis of urea to CO_2 and NH_3, which is of specific interest because urea is an important N fertilizer. Urease is released from living and disintegrated microbial cells, and in the soil it can exist as an extracellular enzyme absorbed on clay particles or encapsulated in humic complexes. Phosphatases catalyses the hydrolysis of both organic phosphate (P) esters and anhydrides of phosphoric acid into inorganic P. Phosphatase activity may originate from the plant roots (and associated mycorrhiza and other fungi), or from bacteria [31].

The objective of this study was to determine the short-term (two year) effects of conservation management practices, such as organic fertilizers on microbiological soil quality indicators in a wheat field under Mediterranean conditions in Kurdistan province of Iran.

II. Material and methods

This research was conducted at the Agricultural Research Center of Sanandaj (35º16′ lat. N; 47º1′ long. E, 1405 m above sea level) in Kurdistan province of Iran in 2008-09 and 2009-10 growing seasons. The dominant soil type is Inceptisol. The annual temperature averages 18 and 21°C and the annual rainfall averages 512 and 534 mm in first and second year respectively. Experiments were arranged in complete block design with three replications. Main plots consisted of six strategies of supplying the basal fertilizer requirements of wheat, including (N1): 20 t farmyard manure ha^{-1}; (N2): 10 t compost ha^{-1}; (N3): 100 kg triple super phosphate ha^{-1} + 250 kg Urea ha^{-1}; (N4): 10 t farmyard manure ha^{-1} + 5 t compost

ha^{-1}, (N5): 10 t farmyard manure ha^{-1} + 5 t compost ha^{-1} + 50 kg triple super phosphate ha^{-1} + 125 kg Urea ha^{-1} and (N6) Control (without fertilizer). Expectation values of basal fertilizers were determined according to soil test analysis. Soil texture was clay loam (28% sand, 42% clay and 30% silt) with 0.8% organic matter and a pH of 7.6. The farmyard manure and compost were also analyzed for chemical and nutrients properties (Table I). Farmyard manure, compost and chemical fertilizers were added to plots before sowing wheat. Urea fertilizer was applied equally two times before sowing wheat and flowering.

TABLE I
CHEMICAL CHARACTERISTICS OF FARMYARD MANURE AND COMPOST APPLIED TO THE SOIL

Characteristic	pH	N	P	K	Ca	Mg	Zn	Cu
		(%)			(ppm)			
Farmyard manure	7.45	0.47	0.49	0.31	2745	1100	8	25
Compost	7.21	0.78	1.15	0.51	1950	1890	43	295

Wheat seeds planted on October 14, 2008 and October 21, 2009. Main plot size was of 15×20 m and spaces between main plots were three meters. The field was irrigated twice with a 7–9 day interval for the better germination of seeds. The field was also irrigated at stemming and flowering along with fertilization, and twice times in grain filling. Weeds removed by hand in all plots.

For soil physical and chemical analyses, soil pH was measured in suspensions with a soil to water (w/w) ratio of 1:2.5. Organic carbon was measured by a colorimetric method with an external heating procedure [1] and total nitrogen in soil was determined using the Kjeldahl method. Soil for microbiological analysis was sampled in wheat plots. Soil samples were collected in crop rhizosphere at flowering stage of wheat growth. Plants were excavated from four random 0.5-m lengths of a row from each plot. Loose soil was shaken off the roots, and the soil that adhered strongly to the roots was carefully brushed from the roots and kept as rhizosphere soil. The four rhizosphere samples from each plot were combined, passed through a 2-mm sieve and stored at 4 °C until required for analysis.

The MBC was determined on a 15-g oven-dry equivalent field-moist soil sample (sieved to <5mm) by the chloroform fumigation extraction method [34]. In brief, organic C from the fumigated (24 h) and non-fumigated (control) soil were quantified by a TOC/TN analyzer (Model: TOC-Vcpn and TNM-1, Shimadzu Corp., Kyoto, Japan). The non fumigated control values were subtracted from the fumigated values. Biomass C was determined using the following formula: MBC = (C in fumigated soil - C in unfumigated soil)/k, where k = 0.45. Each sample had duplicated analyses, and results are expressed on a moisture-free basis.

Protease (EC 3.4.21-24) activity was determined according to Kandeler [9]. One g field-moist soil was incubated in a rotating water bath for 2 h in 5 ml casein solution (2%, w/v) and 5 ml 0.05 M Tris buffer (pH 8.1) at 50 8C. The reaction was stopped with 5 ml 0.92 MTCA. Folin- Ciocalteu's reagent was added to form a colored complex with the aromatic amino acids formed during the incubation, and the absorbance was determined at 700 nm (Perkin Elmer Lambda 25 UV/VIS). To measure alkaline (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) enzymes p-nitro phenyl phosphate disodium (0.115 M) were used as the substrate [17]. Soil samples (1 g) were treated with 2ml of 0.5 M sodium acetate buffer with a pH of 5.5 (using acetic acid) [21] and 0.5 ml of substrate and were incubated at 37 °C for 90 min. Cooling at 2 °C for 15 min inhibited the reaction. The treated samples were then mixed with 2 ml of 0.5M NaOH and 0.5 ml of 0.5 M $CaCl_2$ (to inhibit the enzyme reaction) and centrifuged at 4000 rpm for 5min. Using spectrometry at 398 nm the produced p-nitro phenol was measured [30]. Urease (EC 3.5.1.5) activity was measured using 0.5 M urea as a substrate in 0.1 M phosphate buffer at pH 7.1 [20]. The NH_4^+-N produced by urease activity was determined using a flow injection analyzer (FIAStar, Tecator, S). To account for the NH_4^+-N fixation by soils, NH_4^+-N solutions with concentrations in the range of those released by urease activity was incubated with these spoils. Dehydrogenase activity was determined by the reduction of tri phenyl tetrazolium chloride (TTC) to tri phenyl formazan (TPF) as described by Serra-Wittling *et al*. [28] with modifications. Briefly, moist soil (2 g) was treated with 2.5 ml of 1% TTC-Tris buffer (pH 7.6), and then incubated at 37 °C in darkness for 24 h. All enzyme activities values were calculated based on of oven-dry (105 °C) weight of soil.

Using SAS [26] data were subjected to analysis of variance, including combined analysis. Analysis of variance (ANOVA) was used to detect the treatment effects on measured variables, and the least significant difference (LSD) were used to compare means of measured enzyme activities and microbial biomass carbon ($P < 0.05$). In addition correlation coefficients among soil enzymes and MBC were also determined.

III. RESULTS AND DISCUSSION

The results indicated statistically significant ($p < 0.05$) differences in the level of MBC in the soil between various methods of fertilization. There were no significant differences between interaction effects of fertilization on MBC. The pattern of variation of MBC in the soil during the two years of study was similar. The addition of compost or farm yard manure (FYM), significantly ($p < 0.05$) increased the soil MBC in comparison to the chemical fertilizer and the control. Higher levels of MBC in compost treated soil could be due to greater amounts of biogenic materials like mineralizable nitrogen, water soluble carbon and carbohydrates. Integrated use of chemical fertilizers and organic fertilizers (N5) brings in more MBC in soil compared to their single application of them (Table II). Similar observations were recorded by Leita *et al*. [12]. Fertilizers may meet up the demand of mineral nutrition required by the microbes but not that of carbon, which is a major component of microbial cells. Integrated application of organic and inorganic materials provides a balanced supply of mineral nutrients as well as carbon.

The activities of all enzymes varied significantly in different fertilization methods. The pattern of variation of enzyme activity in the soil during the two years of study was similar; however, urease activity was higher in the first year. The activities of all enzymes were generally higher in the N4 treatment than in the unfertilized and chemical fertilizer treatments (Table II). There were no differences in phosphatase activity between the compost treatment and the FYM treatments. The dehydrogenase, phosphatase and urease activities in the N3 treatment were significantly lower than in the FYM and compost treatments. As shown in table II, alkaline and acid phosphatase generally increased with compost application. Increased phosphatase activity could be responsible for hydrolysis of organically bound phosphate into free ions, which were taken up by plants. Tarafdar and Marschner [31] reported that plants can utilize organic P fractions from the soil by phosphatase activity enriched in the soil– root interface. The observed increase in enzymatic activities due to organic fertilizers amendments are in accordance with previous studies. Martens *et al*. [19] reported that addition of the organic matter maintained high levels of phosphatase activity in soil during a long term study. Giusquiani *et al*. [5] reported that phosphatase activities increased when compost was added at rates of up to 90 t ha^{-1} and the phosphatases continued to show a linear increase with compost rates of up to 270 t ha^{-1} in a field experiment. Application of nitrogen fertilizers significantly decreased urease activity while addition of organic manure increased its activity. The authors concluded that because the nitrogen fertilizers used in the experiments contained NH^{4+} and that the reaction products of urease being NH^{4+}, microbial induction of urease activity had been inhibited. The effect of organic amendments on enzyme activities is probably a combined effect of a higher degree of stabilization of enzymes to humic substances and an increase in microbial biomass with increased soil carbon concentration [19, 4]. This is also indicated by the strong correlation of protease, acid phosphatase and urease with microbial soil C concentrations. Only alkaline phosphatase activity showed statistically non-significant, correlations with MBC (Table III). Compost application increased dehydrogenase activity (Table II). Stronger dehydrogenase activity in compost applied plots may be due to higher organic matter content [33].

Marinari *et al*. [18] reported that a higher level of dehydrogenase activity was observed in soil treated with compost and farmyard manure compared to soil treated with mineral fertilizer. The enzyme activity in organic amendment soil increased by an average 2-4-fold compared with the un-amended soil. Application of compost caused a significant increase in dehydrogenase activity [19]. These results were similar to our finding that dehydrogenase in rhizosphere soil of N2 treatments was average three times higher than that of mineral fertilizer (N3) treatments. In addition, the higher organic matter levels in the compost treatments may provide a more favorable environment for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes. Soil factors, including redox potential (Eh) and pH can affect the rate of enzyme mediated reactions by influencing the redox status and ionization respectively, as well as solubility of enzymes, substrates and cofactors. In addition, some enzymes may predominate at specific pH levels. Application of compost and FYM caused a faster and higher reduction of soil, and at the same time increased the soil pH. Report of Nayak *et al*. [22]

TABLE II
EFFECT OF FERTILIZATION METHODS ON MBC AND SOIL ENZYME ACTIVITY

Treatment	MBC (µg)	Protease (µg)	Acid phosphatase (µg)	Alkaline phosphatase (µg)	Urease (µg)	Dehydrogenase (µg)
Basal fertilizer						
FYM (N1)	278.4 c	86.5 c	167.4 b	2987.3 b	49.6 a	60.1 b
Compost (N2)	312.6 c	94.6 bc	169.2 b	3001.4 b	44.4 b	62.9 ab
Chemical fertilizer (N3)	196.3 d	87.1 c	158.1 c	2678.6 c	28.8 c	21.2 d
FYM + Compost (N4)	409.5 b	110.3 a	226.6 a	3314.4 a	49.8 a	63.8 a
FYM+Compost+Chemical (N5)	691.2 a	96.2 b	169.2 b	2879.1 bc	29.4 c	53.7 c
Control (N6)	89.3 e	73.1 d	41.8 d	2658.7 c	27.9 c	20.8 d

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability.

TABLE III
CORRELATION COEFFICIENTS BETWEEN ENZYME ACTIVITY AND MICROBIAL BIOMASS CARBON

	MBC	Protease	Acid phosphatase	Alkaline phosphatase	Urease	Dehydrogenase
MBC	1					
Protease	0.963 **	1				
Acid phosphatase	0.982 **	0.695 **	1			
Alkaline phosphatase	0.219 ns	0.682 **	0.883 **	1		
Urease	0.882 **	0.133 ns	0.598 **	0.432 ns	1	
Dehydrogenase	0.901 **	0.913 **	0.789 **	0.874 **	0.712 **	1

ns and **: not significant, significant at 1% of probability, respectively.

showed that soil pH was lowest in the inorganic fertilizers amended plots and highest in compost amended plots. Soil dehydrogenase activity exhibited a strong negative relationship with Eh and a positive relationship with Fe^{2+} content, suggesting aeration status is the major factor determining the activity [33].

IV. Conclusion

The present study provides information on soil microbial biomass and activities as influenced by fertilization in wheat production conditions. The results demonstrate that microbial biomass and soil enzyme activity is sensitive in discriminating between organic fertilizers and inorganic fertilizer application on a short-term basis. Soil microbial biomass and enzymatic properties were also closely related with the C inputs.

References

[1] Anderson, J.M., and J.S. Ingram, 1993. Tropical Soil Biology and Fertility. A handbook of methods. CAB International, Wallingford, UK.
[2] Biederbeck, V.O., R.P. Zentner., and C.A. Campbell, 2005. Soil microbial populations and activities as influenced by legume green fallow in a semiarid climate. Soil Biology and Biochemistry, 37: 1775-1784.
[3] Chander, K., S. Goyal., D.P. Nandal., and K.K. Kapoor, 1998. Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. Biology and Fertility of Soils, 27: 168-172.
[4] Crecchio, C., M. Curci., R. Mininni., P. Ricciuti., and P. Ruggiero, 2001. Short term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biology and Fertility of Soils, 34: 311-318.
[5] Giusquiani, P.L., G. Gigliotti., and D. Businelli, 1994. Long-term effects of heavy metals from composted municipal waste on some enzyme activities in a cultivated soil. Biology and Fertility of Soils, 17: 257-262.
[6] Haj Abbasi, M.A., and A. Hemmat, 2000. Tillage impacts on aggregate stability and crop productivity in clay-loam soil in central Iran. Soil and Tillage Research, 56: 205-212.
[7] Hemmat, H., and I. Eskandari, 2006. Dryland winter wheat response to conservation tillage in a continuous cropping system in northwestern Iran. Soil and Tillage Research, 86: 99-109.
[8] Jin, K., S. Sleutel., D. Buchan., S. De Neve., D.X. Cai., D. Gabriels., and J.Y. Jin, 2009. Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. Soil and Tillage Research, 104: 115-120.
[9] Kandeler, E, 1996. Protease activity. In: Schinner F, Ohlinger R, Kandeler E, Margesin R (Eds Methods in Soil Biology, Springer, Berlin/Heidelberg/New York) pp. 165-168.
[10] Kandeler, E., D. Tscherko., and H. Spiegel, 1999. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. Biology and Fertility of Soils, 28: 343-351.
[11] Karlen, D.L., and D.T. Gooden, 1987. Tillage systems for wheat production in the southeastern Coastal Plains. Agronomy Journal, 79: 582-587.
[12] Leita, L., M. De Nobilli., C. Mondini., G. Muhlbacova., L. Marchiol., G. Bragato., and M. Contin, 1999. Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. Biology and Fertility of Soils, 4: 371-376.
[13] Li, C.H., B.L. Ma., and T.Q. Zhang, 2002. Soil bulk density effects on soil microbial populations and enzyme activities during the growth of maize (Zea mays L.) planted in large pots under field exposure. Canadian Journal of Microbiology, 82: 147-154.
[14] Lundquist, E.J., L.E. Jackson., K.M. Scow., and C. Hsu, 1999. Changes in microbial biomass and community composition and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry, 31: 221-236.
[15] Lynch, J.M, 1983. Soil Biotechnology. Blackwell, Oxford, 191 pp.
[16] Madejon, E., F. Moreno., J.M. Murillo., and F. Pelegrn, 2007. Soil biochemical response to long-term conservation tillage under semi-arid Mediterranean conditions. Soil and Tillage Research, 94: 346-352.
[17] Mandal, A., A.K. Patra., D. Singh., A. Swarup., and R.E. Masto, 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology, 98: 3585-3592.
[18] Marinari, S., G. Masciandaro., B. Ceccanti., and S. Grego, 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresoruce Technology, 72: 9-17.
[19] Martens, D.A., J.B. Johanson., and J.W.T. Frankenberger, 1992. Production and persistence of soil enzymes with repeated addition of organic residues. Soil Science, 153: 53-61.
[20] Nannipieri, P., B. Ceccanti., S. Cervelli., and P. Sequi, 1974. Use of 0.1 M pyrophosphate to extract urease from a podzol. Soil Biology and Biochemistry, 6: 359-362.
[21] Naseby, D.C., and J.M. Lynch, 1997. Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of Pseudomonas fluorescens on wheat seed, Soil Biology and Biochemistry, 29: 1353-1362.
[22] Nayak, D.R., Y.J. Babu., and T.K. Adhya, 2007. Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biology and Biochemistry, 39: 1897-1906.
[23] Nishio, M., and S. Kusano, 1980. Fluctuation patterns of microbial numbers in soil applied with compost. Soil Science. Plant Nutrition, 26: 581-593.
[24] Peltzer, S.C., A.I.A. Hashem., B.V.A. Osten., C.M.L. Gupta., D.A.J. Diggle., E.G.P. Riethmuller., F.A. Douglas., G.L.M. Moore., and H. Koetz, 2009. Weed management in wide-row cropping systems: a review of current practices and risks for Australian farming systems. Crop and Pasture Science, 60: 395-406.
[25] Rice, P.J., L.L. McConnell., L.P. Heighton., A.M. Sadeghi., A.R. Isensee., J.R. Teasdale, A.A. Abdul-Baki., J.A. Harman-Fetcho., and C.J. Hapeman, 2001. Runoff loss of pesticides and soil: a comparison between vegetative mulch and plastic mulch in vegetable production systems. Journal of Environment Quality, 30: 1808– 1821.
[26] SAS Institute, 2003. The SAS system for windows. Release 9.1, SAS Inst, Cary, NC.
[27] Schillinger, W. F, 2005. Tillage method and sowing rate relations for dryland spring wheat, barley, and oat. Crop Science, 45: 2636-2643.
[28] Serra-Wittling, C., S. Houot., and E. Barriuso, 1995. Soil enzymatic response to addition of municipal solid-waste compost. Biology and Fertility of Soils, 20: 226-236.
[29] Shams Abadi, H.A., and S. Rafiee, 2007. Study on the effect of tillage practices and different seed densities on yield of rainfed wheat. Iranian Journal of Agricultural Science and Natural Resource, 13: 95-102.
[30] Tabatabai, M.A., and J.M. Bremner, 1969. Use of p-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biology and Biochemistry, 1: 301-307.
[31] Tarafdar, J.C., and H. Marschner, 1994. Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biology and Biochemistry, 26: 387-395.
[32] Tarkalsona, D.D., G.W. Hergertb., and K.G. Cassman, 2006. Long-term effects of tillage on soil chemical properties and grain yields of a dryland winter wheat sorghum-corn-fallow rotation in the Great Plains. Agronomy Journal, 98: 26-33.
[33] Wlodarczyk, T., W. Stepniewski., and M. Brzezinska. 2002. Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from Cambisols under flooding conditions. Biology and Fertility of Soils, 36: 200-206.
[34] Wu, J., R.G. Joergensen., B. Pommerening., R. Chaussod., and P.C. Brookes, 1990. Measurement of soil microbial biomass C by fumigation extraction an automated procedure. Soil Biology and Biochemistry, 22: 1167-1169.