

## Soil Microbial Activity and Biomass as Influenced by Tillage and Fertilization in Wheat Production

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**Abstract:** Tillage systems and fertilization play an important role in crop growth and soil improvement. This study was conducted to determine the best tillage and fertilization system for wheat production. Experiments were arranged in a split plot based on randomized complete block design with three replications in 2008-09 and 2009-10 growing seasons. Main plots consisted of no tillage (T1), minimum tillage (T2) and conventional tillage (T3). 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. Addition of compost or farm yard manure significantly increased soil microbial biomass carbon in comparison with chemical fertilizer. No tillage system increased microbial biomass carbon compared to other tillage systems. The dehydrogenase, phosphatase and urease activities in the N3 treatment were significantly lower than in the farm yard manure and compost treatments. Urease activity under T1N4 treatment in the two years of our study was the highest of all treatments. N5 treatment had a significant difference with other treatments. The highest rate of grain yield was produced in minimum tillage system.

**Key words:** Enzyme activity % Fertilization % Microbial biomass carbon % Wheat % Tillage

### 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 [1]. 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 [2]. 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 [3], biomass of bacteria and fungi [4], enzyme activities [5] and biochemical properties [6]. Microbial communities perform necessary ecosystem

services, including nutrient cycling, pathogen suppression, stabilization of soil aggregates and degradation of xenobiotics. 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 [7].

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 [5]. 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<sub>2</sub> and NH<sub>3</sub>, 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 [8].

The objective of this study was to determine the short-term (two year) effects of conservation management practices, such as no-tillage, reduced tillage and organic fertilizers on microbiological soil quality indicators in a wheat field under Mediterranean conditions in Kurdistan province of Iran.

### MATERIALS AND METHODS

**Site Description and Experimental Design:** This study was conducted at the Agricultural Research Center of Sanandaj (35°16' lat. N; 47°1' 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 the split plot based on randomized complete block design with three replications. Main plots consisted of no tillage (T1), minimum tillage (disk harrowing with average depth of 15 cm + one shallow disk harrowing) (T2) and conventional tillage (moldboard plowing with average depth of 30 cm + two shallow disks followed by secondary tillage with a soil grubber and harrow for seedbed preparation) (T3). In NT, crop residues cut by the combine were chopped and spread evenly with a combine-attached chopper. NT plots were seeded with a NT seed drill. Sub-plots were six strategies of supplying the basal fertilizer requirements of wheat, including (N1): 20 t farmyard manure ha<sup>-1</sup>; (N2): 10 t compost ha<sup>-1</sup>; (N3): 100 kg triple super phosphate ha<sup>-1</sup> + 250 kg Urea ha<sup>-1</sup>; (N4): 10 t farmyard manure ha<sup>-1</sup> + 5 t compost ha<sup>-1</sup>, (N5): 10 t farmyard manure ha<sup>-1</sup> + 5 t compost ha<sup>-1</sup> + 50 kg triple super phosphate ha<sup>-1</sup> + 125 kg Urea ha<sup>-1</sup> 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 1). Farmyard manure, compost and chemical fertilizers were added to plots before sowing wheat. For CT and MT chemical fertilizer or organic fertilizers was applied and then incorporated with tillage, while for NT treatments, fertilizers were surface applied on the plots. Urea fertilizer was applied equally two times before sowing wheat and flowering.

Wheat seeds planted on October 14, 2008 and October 21, 2009. Main plot area 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.

**Soil Sampling:** 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 [9] 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.

**Microbial Biomass Carbon (MBC):** The MBC was determined on a 15-g oven-dry equivalent field-moist soil sample (sieved to <5mm) by the chloroform fumigation extraction method [10]. 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:  $MBC = (C \text{ in fumigated soil} - C \text{ in unfumigated soil})/k$ , where  $k = 0.45$ . Each sample had duplicated analyses and results are expressed on a moisture-free basis.

Table 1: 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

**Soil Enzyme Activities:** Protease (EC 3.4.21-24) activity was determined according to Kandeler [11]. One gram 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 °C. The reaction was stopped with 5 ml 0.92 M TCA. 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 [12]. Soil samples (1 g) were treated with 2ml of 0.5 M sodium acetate buffer with a pH of 5.5 (using acetic acid) [13] 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<sub>2</sub> (to inhibit the enzyme reaction) and centrifuged at 4000 rpm for 5min. Using spectrometry at 398 nm the produced p-nitro phenol was measured [14]. 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 [15]. The NH<sub>4</sub><sup>+</sup>-N produced by urease activity was determined using a flow injection analyzer (FIASStar, Tecator, S). To account for the NH<sub>4</sub><sup>+</sup>-N fixation by soils, NH<sub>4</sub><sup>+</sup>-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.* [16] 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 oven-dry (105°C) weight of soil.

**Statistical Analysis:** Using SAS [17] 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.

## RESULTS AND DISCUSSION

**Microbial Biomass-C in Soil:** The results indicated statistically significant ( $p < 0.05$ ) differences in the level of MBC in the soil between various methods of tillage and fertilization. There were no significant differences between the interaction effect between tillage and fertilization on MBC. The pattern of variation of MBC in the soil during the two years of study was similar. Addition of compost or farm yard manure (FYM), significantly ( $p < 0.05$ ) increased soil MBC in comparison with chemical fertilizer or 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 2). Similar observations were recorded by Leita *et al.* [18]. 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.

NT system increased MBC compared to other tillage systems (Fig. 1). Conventional tillage decreased soil organic matter and soil structure may be due to the reduction in soil microbial communities. Madejon *et al.* [19] observed that conservation tillage increased MBC and microbial activities. Along with microbial biomass changes, one might also expect shifts in microbial community structure to occur due to the temporal increase in microbial niche, water retention or reduced physical disturbance with no-tillage.

Table 2: 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.

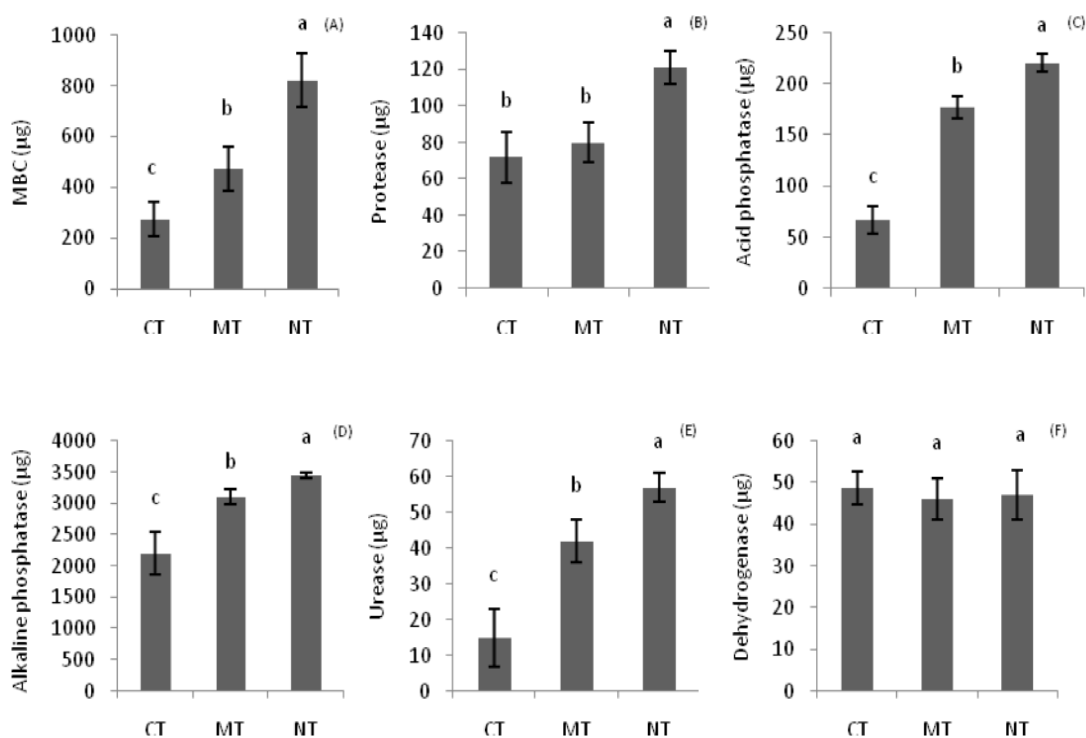


Fig. 1: Effect of tillage practices on MBC (A), protease (B), acid phosphatase (C), alkaline phosphatase (D), urease (E) and dehydrogenase (F) activity in soil.

**Soil Enzyme Activities:** The activities of all enzymes varied significantly in different fertilization methods. Only, urease activity was significantly affected by the two-way interactions of fertilizers  $\times$  tillage. 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 2). 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 2, 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 [8] 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.* [20] reported that addition of the organic matter maintained high levels of phosphatase activity in soil during a long term study. Giusquiani *et al.* [21]

reported that phosphatase activities increased when compost was added at rates of up to 90 t haG<sup>1</sup> and the phosphatases continued to show a linear increase with compost rates of up to 270 t haG<sup>1</sup> 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<sup>4+</sup> and that the reaction products of urease being NH<sup>4+</sup>, 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 [20,21]. 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 3). Compost application increased dehydrogenase activity (Table 2). Stronger dehydrogenase activity in compost applied plots may be due to higher organic matter content [23]. Marinari *et al.* [24] 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.

Table 3: 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.

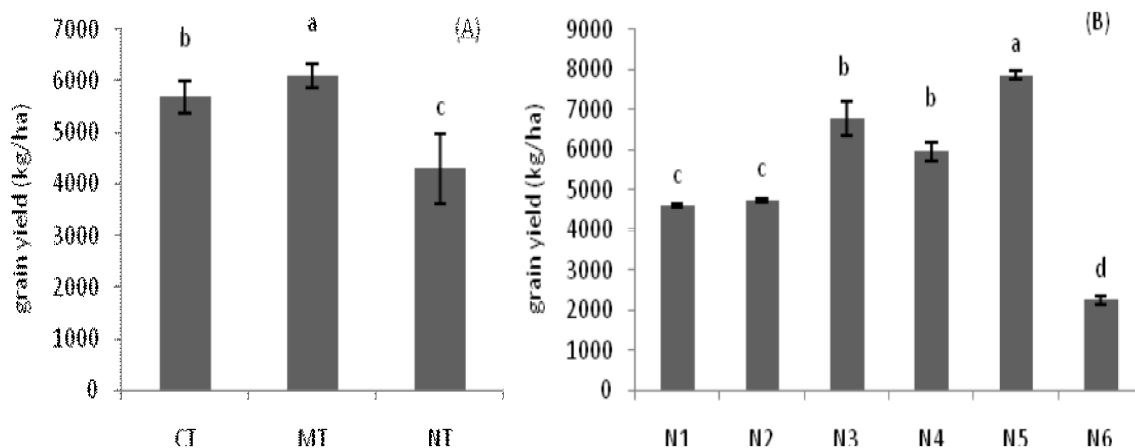


Fig. 2: Effect of tillage practices (A) and fertilization (B) on wheat grain yield

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 [20]. 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 highest 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.* [25] 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 [23].

Results indicated statistically significant ( $p < 0.05$ ) differences in the enzyme activity in the soil between various methods of tillage. However dehydrogenase activity does not affected by tillage methods. The activity of acid, alkaline phosphatase and protease tended to be higher in the NT treatment compared to the MT and CT treatments. However, activity of urease and dehydrogenase were similar in NT and MT treatments (Fig 1). Finding of Jin *et al.* [26] has already suggested the positive effects of conservation tillage practices on soil enzyme activities. The generally higher enzyme activities in NT mainly resulted from the larger water availability in the plots rather than the better soil fertilities. Urease activity under T1N4 treatment in the two years of our study was the highest of all treatments. In this treatment co-application of compost and farmyard manure in no tillage system assemble good condition for urease activity. The higher bulk density could account for this difference. Enzyme activities were shown to be linearly related to soil bulk density [27].

**Grain Yield:** Wheat grain yield was affected by different fertilization systems. Fertilizers comparison revealed that N5 treatment had a significant difference with other treatments (Fig. 2). For justification of this difference it could be stated that parallel to meeting plant need to

phosphorus and nitrogen, adding compost and farmyard manure to soil can provide micro elements for plant. Compost applied in the current study has been shown to contain elevated concentrations of micro elements including zinc (Zn). Also, it seems that organic fertilizers causes improving soil structure and optimizing root growth conditions by providing organic matter and nutrients. The pattern of variation of grain yield during the two years of study was similar

Tillage systems and cultivars significantly affected grain yield. The highest rate of grain yield was produced in MT that was statistically greater than which in CT and NT systems (Fig. 2). Applying CT system caused to a reduction of 8 percentages in grain yield as compared with MT. Since CT system cause to soil moisture reduction, therefore this tillage system is not recommended under dry framing conditions. Increasing cone index and soil compaction are the main reasons of yield reduction. Soil compaction leads to restriction of root growth. Consequently water and nutrient uptake by roots will be confused and diminished. Schillinger [28] demonstrated that using NT system caused to lower production of wheat, barley and oat in comparison with CT system. Main reasons cited for lower yields under no-till system are reductions in plant density [29], increased weed infestation [30] and soil physical properties that limit crop growth [31]. The most probable cause of erratic stand establishment for no-till wheat treatment was poor soil-seed contact associated with the use of the drill for seeding into a layer of crop residue [32]. On the other hand, Tarkalsona *et al.* [33] reported that application of NT system in a long term period led to indicative improvement in wheat productivity in comparison with CT system. No tillage system needs specific planting tools that cannot be find easily in Iran, therefore using MT is more favored by farmers. Shams-Abadi and Rafiee [34] resulted that using MT, leads to increase wheat production.

### CONCLUSION

The present study provides information on soil microbial biomass and activities as influenced by fertilization and tillage 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. Consistent distinctions in enzyme activities were

observed between different tillage practices. These differences were most pronounced between no tillage at the one hand and conventional and reduced tillage at the other hand. The yield of wheat was impacted by soil fertility amendments and tillage methods in our study. Organic manure increased yield by altering soil physical properties, increasing soil fertility, increasing beneficial microbial populations and activity.

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